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AMERICAN DAIRY SCIENCE ASSOCIATION

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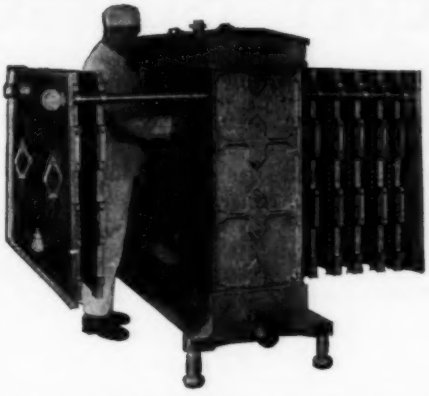

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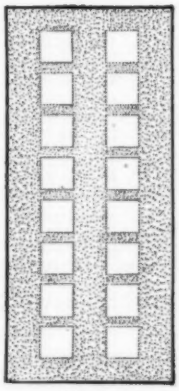

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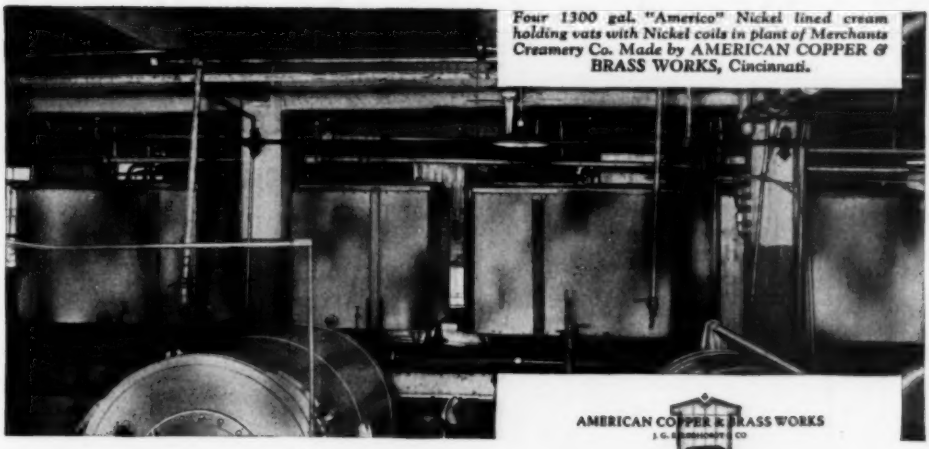

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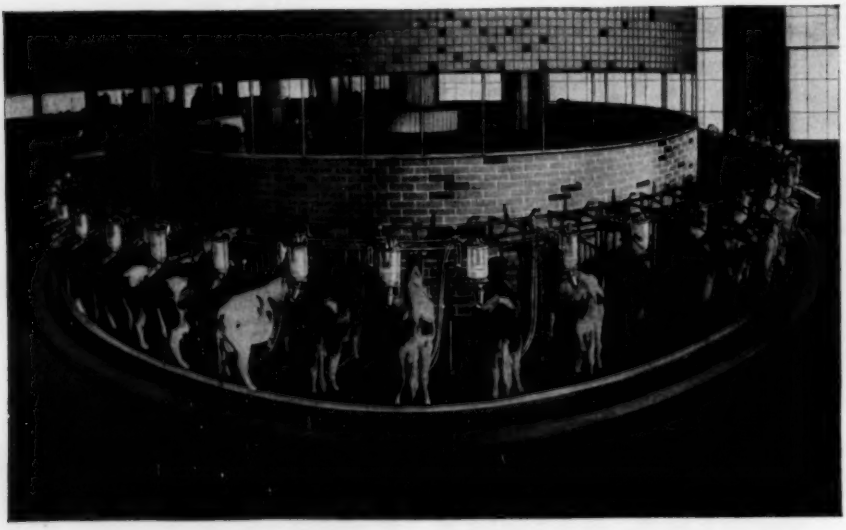

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THE EFFECT OF THE HYDROGEN ION CONCENTRATION ON THE CHURNING TIME OF CREAM*

E. S. GUTHRIE AND PAUL FRANCIS SHARP

Department of Dairy Industry, Cornell University, Ithaca, N. Y.

INTRODUCTION

Those familiar with churning generally recognize that as the acidity in cream develops, the time required for churning decreases. This increase in acidity, due to natural souring, covers only a rather narrow range of pH; namely, from about 6.8 to 4.4. It is probable that in certain cases cream may be neutralized, so that churnings might be carried out at a pH slightly above 7. As long ago as 1879, Clausnizer (2) reported that cream from milk to which considerable amounts of alkali had been added, could be churned; Babcock (1) confirmed this finding.

Ruehe and Stiritz (3) used the fat loss in the buttermilk as an index of various factors affecting the stability of cream. Their experiments were with neutralized cream. They found that the addition of 1 per cent of sodium chloride and of hydrochloric acid in an amount sufficient to increase the acidity by 0.05 per cent expressed as lactic acid, decreased the fat lost in the buttermilk. A combination of both sodium chloride and hydrochloric acid was most effective.

Van Dam and Holwerda (4) diluted ripened cream with sour milk plasma and milk serum in such proportions that increasing amounts of casein resulted. In one series the creams ranged in pH from 4.47 to 4.50. The churning time at first decreased slightly with an increase in casein, and then increased again with the larger amounts of casein. The fat loss in the buttermilk decreased uniformly as the amount of casein increased. They (5) also studied the effect of decreasing the concentration of the water soluble constituents by diluting cream with either milk

* Presented at the Madison, Wisconsin, Meeting of the American Dairy Science Association June 27, 1928. Received for publication May 19, 1930.

serum or water, and found that the dilution of the water soluble constituents increased churning time slightly and decreased the fat loss in the buttermilk. Van Dam and Holwerda (6) studied the influence of acidity as caused by acid developed by ripening. Although the difference in acidity amounted to only a few tenths of a pH (in one series from an average of 4.59 to 4.39), yet a decrease in fat loss was noted from 0.52 to 0.42. Little influence was exerted on the churning time.

Many other investigators have carried out experiments in which the influence of starter and development of acidity were studied. So far as we were able to discover, no one had studied the effect of pH on churning over a wide range. Such a study was, therefore, undertaken in the hope that some light might be thrown on the mechanism of churning. It was believed that the stability of the cream or the resistance of cream to churning would be most accurately evidenced by the churning time. The effect of pH on the churning time, therefore, was studied.

EXPERIMENTAL

Description of apparatus

In order to carry out such an investigation, it was necessary to control the other factors, besides the pH. Very few churnings have been executed under, what would be considered, accurately controlled experimental conditions. Since cream is usually changing in its churning properties, the carrying out of a series of churning experiments, one after the other in the same churn, may not give results which are exactly comparable with each other, due to such factors as the change in acidity, aging of the cream, and change in the proportion of liquid and solid fat in the fat globules. In addition, the temperature of the cream before and during the churning process may not be adequately controlled.

In our attempt to perform controlled churning experiments, 4 one-gallon glass churns containing agitators were so arranged that the glass parts of the churns were almost completely immersed in a water thermostat which was vigorously stirred. These 4 churns were all driven from the same line shaft by a relatively powerful electric motor. The driving mechanism was

composed entirely of gears so that there was no chance for one churn to run more slowly than the others or the whole set to run more slowly one time than another. The outfit as shown in figure 1, was so arranged that a churn could be removed or started at will without stopping any of the others. The motor was powerful enough so that the removal or addition of the churns produced no appreciable effect upon its speed. In all of the work reported here the churnings were carried out in sets of four,

FIG. 1. APPARATUS FOR EXPERIMENTAL CHURNING INVESTIGATIONS

and in every case one churn was operated as a control as a basis for comparison of the variable factors which were being studied in the other three. Before the motor was started, the cream was placed in the churns and the churns were set in the water bath for one hour to permit the cream to come to the churning temperature. The cream had previously been held at 2°C. for at least twelve hours. The water thermostat was kept at 17°C. (62.6°F.)

The procedure which was used in adjusting the cream to the various hydrogen ion concentrations was as follows: Cream from fresh milk was separated, standardized to 40 per cent fat, and pasteurized. To 1125 gms. of this sweet cream were added 375 cc. of water or solution, giving as a result cream of 30 per cent fat content. In the case of the control churning, 375 cc. of

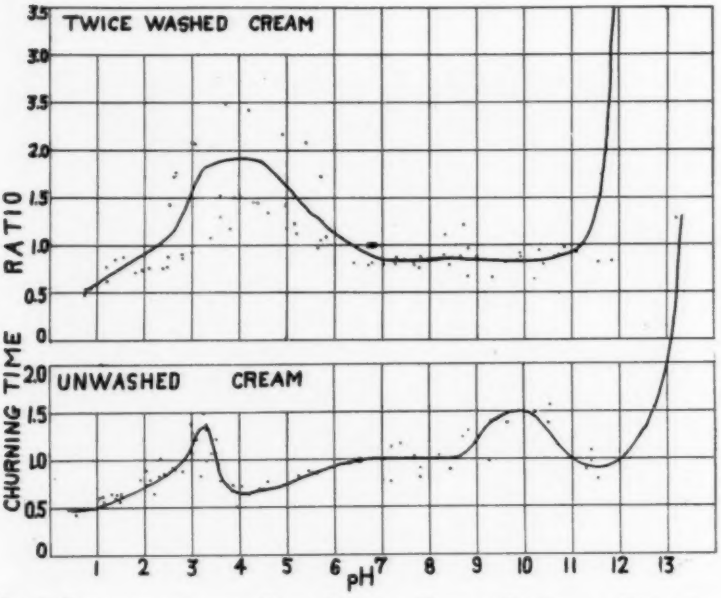

FIG. 2. RELATION BETWEEN pH AND CHURNING TIME OF WASHED AND UNWASHED CREAM

Churning time of the control taken as 1

distilled water were added. To the other three churns 375 cc. of a solution, which contained the appropriate amount of hydrochloric acid, sodium hydroxide, or acid or alkali plus salt to give the pH desired, were added.

It was found that, even with the precautions taken and the similarity in the churns, the four churns did not churn the control cream (1125 grams of sweet cream plus 375 cc. of distilled

water) in the same time, but that some of the churns consistently required more time to churn the same cream than did other churns. Several churnings were made, in which the churns were all filled with the same cream, and from the average results so obtained, ratio-time factors were calculated so that this difference in time required to churn the control sample could be corrected for when the variously treated creams were run in the same churns. One churn was always operated with the control cream.

Churning washed and unwashed cream at various pH values

The results obtained on adjusting the unwashed cream to various hydrogen ion concentrations are shown in figure 2 in which the results are expressed on the basis of the churning time of the control as 1.0; the average pH of the control was about 6.6 to 6.7. The usual churning time of the control was 20 minutes. As the acidity was increased, the churning time decreased until a minimum was reached at about pH 4.2. As the hydrogen ion concentration was increased still further, the churning time increased until a maximum was reached at a pH of a little above 3. From there on the churning time steadily decreased as the hydrogen ion concentration increased. On the addition of alkali to the cream, the churning time at first remained constant and then increased to a maximum at near pH 10. As still more alkali was added, the churning time decreased to a minimum at near pH 11.5, and upon further additions of alkali, the churning time increased fairly rapidly until the curve evidently approached an asymptote of infinite churning time at somewhere near pH 13.5.

This curve brings out at least two interesting points. The first is the fact that churning can be obtained over such an extended range of pH; the second is the apparent relationship of long churning times to dispersion or hydration of the casein and of the short churning times to its precipitation or dehydration. The isoelectric point of casein is near a pH of 4.7, in which region the casein is not in solution and the churning time is at a minimum. As pH 3.0 is approached, the casein is dispersed and the churning time is lengthened. As pH 1 is approached casein again is precipitated and the churning time becomes shorter.

Several investigators of the viscosity of alkaline casein solutions have found a maximum near pH 9 to 10 and our experiments show that in this region the churning time is lengthened. In the highly alkaline solutions, that is pH 12 to 13, we again find a great increase in the churning time which may perhaps be due to soap formation. The effect of alkali on the churning time at the high pH values shows a distinct difference from the effect of acid, at the very low pH values.

Still further information on the factors influencing the churning time was obtained by churning twice washed cream. The cream was first separated to give about 40 per cent fat. To this cream was then added 9 volumes of water and the cream again separated. To the cream so obtained 9 additional volumes of water were added and the separation was again carried out. The resulting cream was standardized to 40 per cent fat with water and is called in figure 2 the "twice washed cream." Such washing should, by straight dilution as calculated, reduce the plasma constituents in the free liquid phase to about 1/250 of their concentration in the unwashed cream. The actual reduction is, however, probably considerably less due to adsorption on the surface of the fat globules and the probable change in the amount adsorbed after each washing. The churnings and the adjustment of the pH were carried out in the same manner as described for unwashed cream. The churning time of the control was about thirteen minutes. The data are presented graphically in figure 2. It will be noted that the curves for the twice washed cream and the unwashed cream are distinctly different. In the case of the twice washed cream as the acidity is increased, there is a distinct increase in churning time up to a maximum in the neighborhood of pH 4.0. From there on as the hydrogen ion concentration increases there is a decrease in churning time. This maximum at pH about 4.0 for the twice washed cream is almost exactly at the same hydrogen ion concentration as the minimum for the unwashed cream. The two sets of points in the acid region where one set is above the curve and the other below are due to the use of two different lots of cream.

As alkali is added to the washed cream there is at first a slight

decrease in churning time, followed by a horizontal portion which indicates that changing the pH through the region from 7 to 10.5 exerts no effect on the churning time. Upon increasing the pH above 11.0 the churning time increases tremendously, possibly approaching infinite churning time at some place near pH 12.5. The pH churning time curve for the unwashed cream is a fairly typical casein curve, while the pH churning time curve for the twice washed cream is distinctly not a casein curve. Since the importance of the casein as a factor in regulating the churning time is indicated by these two curves, other experiments were devised to show its influence.

Churning in the presence of NaCl and $CaCl_2$ at various pH values

When water is made half normal with respect to sodium chloride, casein does not dissolve on the acid side of the isoelectric point, that is near pH 3. If the shortness of the churning time is correlated with separation of the casein from solution, then by making the water of the cream half normal with respect to sodium chloride, there should be no lengthening of the churning time of the unwashed cream at near pH 3.2. Such experiments were carried out and the results are expressed graphically in figure 3. It will be noted that in the neighborhood of pH 3, while there is a very slight bump in the curve, yet the churning time is considerably shorter than the control, indicating that in this case the insolubility of the casein is associated with a short churning time. Approximately the same results were obtained when the water was made tenth normal with respect to sodium chloride. In the cases where the pH of the creams containing sodium chloride were increased, maxima were obtained in the neighborhood of 9.5 and 10, which corresponds to the maximum obtained with cream to which no salt was added. Sodium chloride does not render the casein insoluble on the alkaline side of the isoelectric point. In the presence of the sodium chloride as the pH approached the high alkalinities, the churning time increased to infinity. The break in the curve toward infinity seemed to occur at a lower pH the higher the concentration of sodium chloride.

When the water of the cream was made half normal with

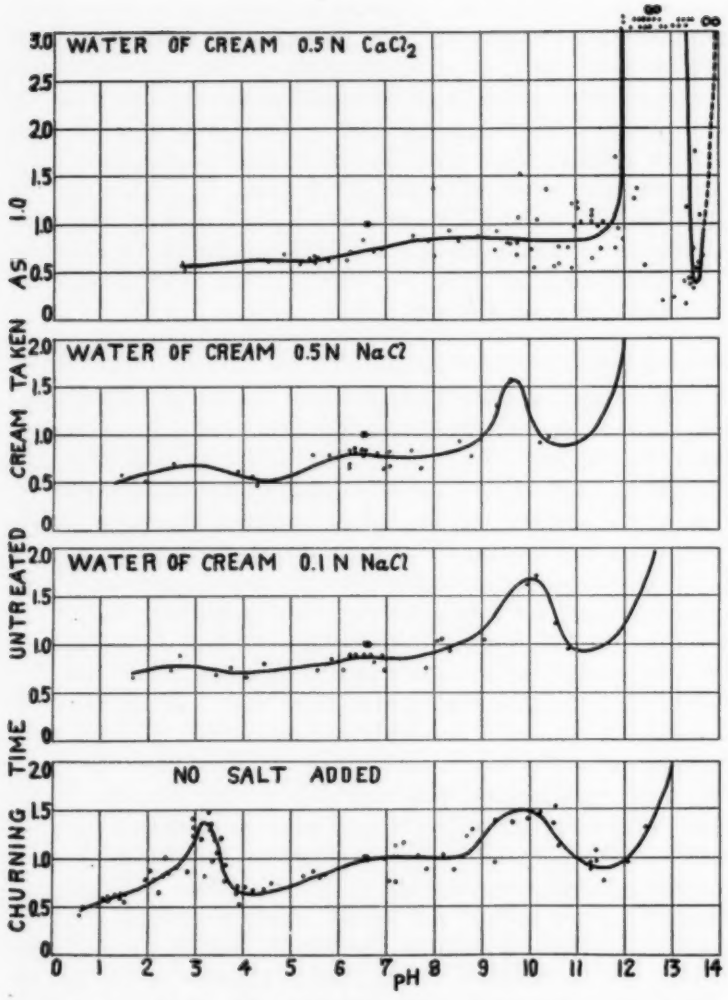
WATER OF CREAM 0.5 N $CaCl_2$
WATER OF CREAM 0.5 N NaCl
WATER OF CREAM 0.1 N NaCl
NO SALT ADDED
CHURNING TIME AS 1.0
UNTREATED CREAM TAKEN
pH

FIG. 3. RELATION BETWEEN pH AND CHURNING TIME OF CREAM TO WHICH SODIUM OR CALCIUM CHLORIDE HAD BEEN ADDED
Churning time of the control taken as 1

respect to calcium chloride on the acid side of the isoelectric point, the results were very similar to those obtained with sodium chloride. Calcium chloride prevents the casein from dispersing near pH 3. The calcium chloride also caused a shortening of the churning time in the region between pH 7.0 and 11.5. In the case of the addition of calcium chloride there was no hump in the curve at pH 10.

In the presence of calcium chloride the churning time approaches infinity (followed for 40 hours in some cases) between pH 12 and 13.3. Above this pH there is a narrow range where the churning time is very short and on the addition of still more alkali the churning time approaches infinity again. The actual pH values could not be determined in the presence of this high concentration of alkali or at least the readings were very erratic and indicated pH values between 8 and 10.

When considerable sodium hydroxide is added to calcium chloride a precipitate of the relatively insoluble calcium hydroxide is formed which results in the formation of considerable sodium chloride in the solution.

$$CaCl_2 + 2NaOH = Ca(OH)_2 + 2NaCl$$

Consequently some of the effects of the addition of sodium chloride might be indicated. The hump in the curve at pH 10 was not in evidence. The churning time did approach infinity at near pH 12 which corresponds to the rise in the curve with 0.5 normal sodium chloride.

The churning times at the high pH values are probably influenced by the hydrolysis of the fat and the formation of soaps. Since soaps tend to reduce the surface tension to a marked degree and alkalies tend to dissolve protein, it is conceivable at least that the material which is naturally adsorbed on the surface of the fat globules might be displaced by the sodium or calcium soaps. If a sodium soap was adsorbed, as might be expected in cases where the cream was simply made highly alkaline or was made alkaline in the presence of sodium chloride, one would expect an extremely stable emulsion, since sodium soaps form and stabilize emulsions of oil-in-water. One would hardly expect

emulsions so stabilized to churn. Calcium soaps, on the other hand, tend to change emulsions of oil-in-water over to emulsions of water-in-oil; therefore, the presence of the calcium hydroxide

TABLE 1

Effect of the degree of dispersion and hydration of the casein on the churning time

EXPERIMENT NUMBER	SUBSTANCE ADDED							CHURNING TIME
	Water	Skim-milk	Grain curd casein solution	Rennet casein suspension	Rennet extract	Sodium flouride	Hydrochloric acid	
1	+				−	−		1.00
	+				+	−		0.70
	+				−	+		1.04
	+				+	+		1.00
2		+			−	−		1.07
		+			+	−		0.51
		+			−	+		1.05
		+			+	+		0.90
3			+	−	−			1.04
			+	−	+			0.47
			−	+	−			0.75
			−	+	+			0.47
4	+	−					−	1.00
	−	+					−	1.07
	+	−					+	0.67
	+	−					+	0.71
5	+	−	−		−	−	−	1.00
	−	+	−		−	−	+	0.93
	−	−	+		−	+	−	1.17
	−	−	+		+	+	−	0.71
6	+			−	−	−		1.00
	+			+	−	−		0.86
	+			+	+	−		0.47
	+			+	+	+		0.70

in the highly alkaline solutions might tend to form calcium soaps and break the emulsion, forming butter. This is a possible explanation of the short churning times found at pH 13.5 when

calcium chloride was added. The reason for the infinite churning time at pH 12 to 13.3 in the presence of calcium chloride is not apparent.

Effect of degree of dispersion of the casein on churning time at pH 6.45 to 6.8

With the exception of the highly alkaline region, the churning time can be more or less correlated with the dispersion of hydration of the casein. In order to gain more information on the effect of casein on churning time, the degree of dispersion of the casein was changed by several combinations obtained by the addition of water, skimmilk, casein, rennet, sodium flouride, and acid. The results obtained are given in table 1.

Each experiment is the average of two or more sets of churnings. The churning time of the control, where only water was added, is given the value 1.00. All of the other churnings have been related either directly or indirectly to this value. The pH values all fell between 6.45 and 6.8, with the exception of the experiments in which hydrochloric acid was added where the pH was 4.4 to 4.6.

The additions listed in table 1 were made as follows: (1) water 375 cc., (2) skimmilk 375 cc., (3) grain curd casein—17 grams dispersed in 375 cc. of $CaCl_2$—NaOH solution to give pH 6.8, (4) rennet casein 17 grams finely ground suspended in 375 cc. of water, (5) rennet extract added casein allowed to coagulate, (6) sodium flouride to make the water of the cream 0.1 normal; in cases where rennet was also present, the rennet was allowed to act before the sodium flouride was added, and (7) enough hydrochloric acid to give a pH 4.4 to 4.6.

In some few cases the relative effects produced when one experiment is compared with another are not quantitatively comparable, yet table 1 does show some general relationship of these treatments. The addition of rennet, which brings the casein out of solution, causes a shortening of the churning time as does the addition of acid. The addition of sodium flouride which is a very effective peptizing agent especially for rennet casein or curd, tends to lengthen the churning time. The addition of the

rennet casein suspension shortens the churning time, while the addition of skimmilk or a solution prepared from grain curd casein lengthens the churning time slightly. The churning time seems to be shortened most of all by the addition of milk or casein followed by the addition of rennet.

Quality of stored butter

The butter made in these various churnings was stored for seven months or longer at 0°F., and was then scored. The main results obtained on the relation of the various quality factors observed are given in figure 4. It will be noted that there are

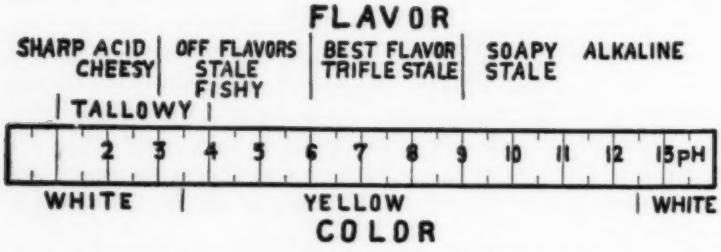

FIG. 4. RELATION BETWEEN pH OF THE CREAM AT THE TIME OF CHURNING TO THE QUALITY OF THE BUTTER AFTER STORAGE

two regions in which the butter became white or bleached. Because a sample was bleached, it did not necessarily follow that it was also tallowy. The most acid samples were white, but were not tallowy. The butter had the best flavor between pH 6 and 9. Several defects made their appearance at pH 3 to 6, the samples being fishy, tallowy, and stale. Below pH 3 the samples tasted of acid and were tallowy down to pH about 1; from there on they did not taste tallowy. Above pH 9 the samples tasted soapy. It should be remembered that while the best flavor was found in the range from pH 6 to 9, the range of best flavor might be quite different if the cream had first been allowed to sour and had then been neutralized.

SUMMARY

The effect of pH on churning time was investigated using cream, washed cream, and cream to which sodium and calcium chloride were added. The results obtained together with the effect of other means of increasing or decreasing the degree of dispersion or hydration of the casein, indicated that hydration or dispersion of the casein was associated with a lengthening of the churning time and that dehydration or precipitation was associated with a shortening of the churning time.

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SIZE OF COW AND EFFICIENCY OF MILK PRODUCTION*

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When we speak of the efficiency of a machine, a process, or an animal, we commonly speak in terms of a ratio of one sort or another. Thus, Jordan (1) in speaking of the efficiency of various farm animals kept for food employs the ratio

$$\frac{\text{pounds of edible solids produced}}{\text{pounds of digestible organic matter consumed}}$$

For the case of the dairy cow the writer (2) has suggested a "coefficient of efficiency" similar in nature to Jordan's ratio. It is based on the known relation between the fat percentage of the milk and the energy or nutritive value of the milk, and, on the known feed requirements for lactation and body maintenance. The formula, based on a year period, is

$$CE = 52.6 \frac{FCM}{FCM + 8.847W}$$

where CE is the coefficient of efficiency, FCM[1] is 4 per cent milk in pounds, and W is live weight of the cow in pounds.

The formula is intended to express the percentage of the feed energy which is recovered as milk energy. The facts required for the estimate of the coefficient are the weight of the cow and the yearly yield of milk and fat. Clearly by the formula CE increases with yield and decreases with weight. It cannot be less than zero and approaches 52.6 as a limit. The limit, 52.6, represents the efficiency of the mammary gland as apart from the body. At a given live weight, say 1,000 pounds, the CE's cor-

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[1] FCM = 0.4 × milk + 15 × fat all in the same unit of weight. One pound FCM = 340 calories. For review of the energy basis of measuring milk yield see Gaines (3).

responding to a few yields (pounds of 4 per cent milk per year) are as follows:

Yield	0	5,000	10,000	15,000	20,000
CE	0	19.5	27.9	33.0	36.5

It is well known that the higher the yield of milk the more economical the production per unit of milk. It is also well known that the larger the cow the higher the yield. These relations are perhaps responsible for the notion that large cows are more efficient than small cows.[2]

If efficiency is defined as above it is clear that the relative efficiency of the small and large cow will depend upon the relation between live weight and milk yield. McDowell (4) has recently published a summary of a large number of records from dairy herd improvement associations in the United States which shows the weight-yield relationship. It is proposed here to adapt his data to the efficiency formula given above.

McDOWELL'S DATA

The yield of 4 per cent milk and coefficient of efficiency have been calculated from McDowell's published figures and are given in table 1. The feed cost per hundred-weight of 4 per cent milk has been computed for purposes of comparison. For the sake of completeness the number of records is also given. Figure 1 shows graphically the computed data, omitting Shorthorns.

Study of table 1 and figure 1 shows that the 4 per cent milk or energy yield increases more or less regularly with size of cow in each of the breed classes. In the case of Guernseys and Ayrshires the yield curves for grade and registered cows are nearly coinci-

[2] A contributing cause is also to be found in the early work of farm management investigators who found in certain whole-milk districts that large cows were more profitable than small cows, and deduced a direct connection between size of cow and economy of production. This deduction overlooked the fact that (for example) the Holstein breed of large cows produces a low-energy milk and the Jersey breed of small cows produces a high-energy milk, while both milks sold at the same price. The large cow was more profitable not because of her size but because her milk sold at a higher price per calorie. This market advantage of low-energy milk is now pretty much a thing of the past in many sections of this country.

TABLE 1

Relation of size of cow to energy yield, calculated biological efficiency, and observed economic efficiency (derived from McDowell's data)

(Cows 5 to 9 years of age)

BREED	LINE*	COWS CLASSIFIED BY WEIGHT, POUNDS										
		600	700	800	900	1000	1100	1200	1300	1400	1500	1600
Grade Jersey	a	193	658	3,491	2,513	2,862	510	249				
	b	5,883	6,430	6,990	7,314	7,528	7,836	8,102				
	c	27.7	26.8	26.1	25.2	24.2	23.5	22.8				
	d	107	101	94	92	90	88	89				
Registered Jersey	a	38	197	1,629	1,222	1,479	235	76				
	b	6,060	6,847	7,257	7,594	7,858	8,023	8,220				
	c	28.0	27.6	26.6	25.7	24.7	23.8	23.0				
	d	111	107	99	97	98	102	100				
Grade Guernsey	a		234	2,091	3,107	7,179	2,252	1,371	149			
	b		6,399	6,825	7,110	7,428	7,679	7,992	7,877			
	c		26.7	25.8	24.8	24.0	23.2	22.6	21.4			
	d		103	91	89	88	89	88	85			
Registered Guernsey	a		19	311	502	1,487	568	341	54			
	b		6,454	6,817	7,161	7,278	7,534	7,723	7,707			
	c		26.8	25.8	24.9	23.7	23.0	22.2	21.1			
	d		105	109	105	106	105	104	109			
Grade Ayrshire	a			42	56	167	95	77	14			
	b			6,603	6,472	6,890	7,562	8,089	8,077			
	c			25.4	23.6	23.0	23.0	22.7	21.7			
	d			97	90	90	91	87	99			

Registered Ayrshire	a			25	39	216	113	77	8			
	b			6,199	6,077	6,942	7,649	7,612	9,195			
	c			24.6	22.8	23.1	23.1	22.0	23.4			
	d			108	118	102	102	106	87			
Grade Holstein	a			1,230	2,666	13,658	10,616	13,933	3,130	1,959	228	87
	b			6,649	7,030	7,437	7,834	8,191	8,629	8,941	9,267	9,128
	c			25.5	24.7	24.0	23.5	22.9	22.5	22.1	21.6	20.6
	d			93	88	86	87	88	87	88	86	87
Registered Holstein	a			89	238	2,061	2,798	6,098	2,560	2,607	644	290
	b			6,874	7,663	7,952	8,327	8,807	9,329	9,549	9,904	10,511
	c			25.9	25.8	24.9	24.3	23.9	23.6	22.9	22.5	22.3
	d			105	100	96	96	95	95	97	98	98
Grade Shorthorn	a			330	632	3,121	1,781	1,897	351	157		
	b			5,806	5,888	6,196	6,465	6,847	7,206	7,318		
	c			23.7	22.4	21.7	21.0	20.6	20.3	19.5		
	d			81	78	77	77	79	79	82		

* Line a, number of records averaged.

Line b, average energy yield per cow per year, pounds 4 per cent milk.

Line c, calculated coefficient of efficiency $= 52.6 \frac{FCM}{FCM + 8.847W}$

Line d, observed feed cost per hundredweight 4 per cent milk, cents

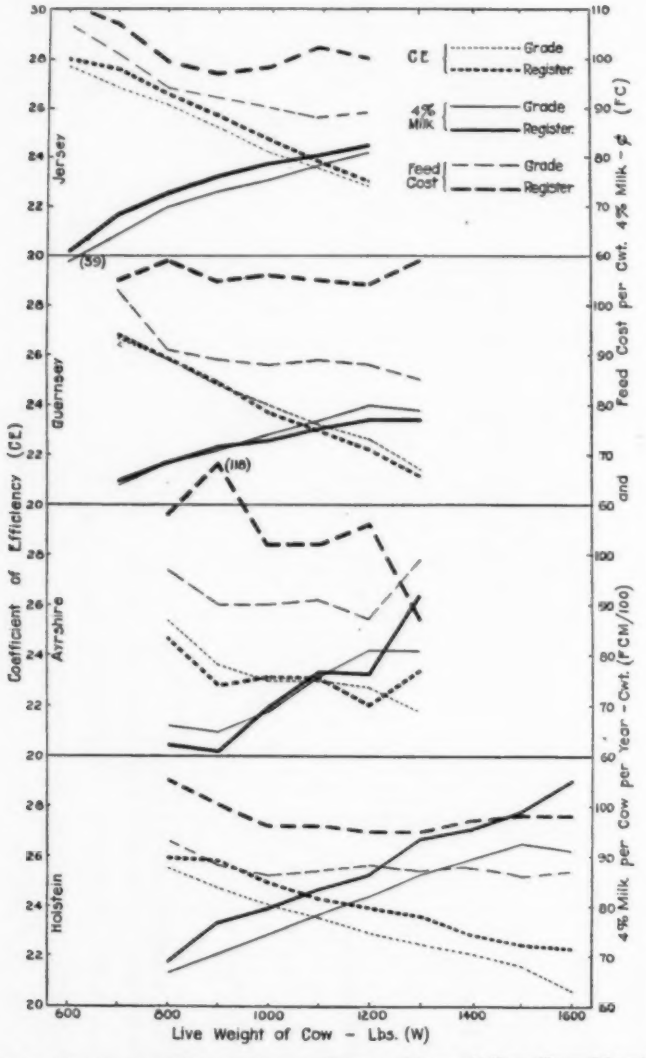

FIG. 1. RELATION OF SIZE OF COW TO ENERGY YIELD (4 PER CENT MILK), THEORETICAL EFFICIENCY, AND RECORDED FEED COST

dent. For Jerseys and Holsteins, on the other hand, the yield of the registered cows is consistently higher than that of grades of the same weight.

The figures for the calculated efficiency show a consistent trend, decreasing as weight increases. That is to say the increase in yield with increase in size is not sufficient to offset the extra feed requirements for maintenance. This holds true in all cases although in the Ayrshire breed the *CE* curve has little slope.

TABLE 2

Relation of size of cow to relative energy yield and efficiency

WEIGHT	NUMBER OF RECORDS	F.C.M. 4 PER CENT MILK PER COW PER YEAR	FEED COST PER HUNDRED-WEIGHT 4 PER CENT MILK	$52.6\frac{CE}{FCM}$ $\frac{FCM}{FCM+8.847W}$	$52.6\frac{CE'}{FCM}$ $\frac{FCM}{FCM+88.47W^{1/3}}$
pounds		*pounds*	*cents*		
600	231	6,284	104	28.5	26.3
700	1,108	6,874	99	27.7	26.1
800	9,238	7,236	92	26.6	25.6
900	10,975	7,416	90	25.4	24.9
1,000	32,230	7,539	89	24.2	24.2
1,100	18,968	7,736	90	23.3	23.7
1,200	24,119	8,016	90	22.6	23.4
1,300	6,266	8,359	90	22.1	23.3
1,400	4,723	8,548	91	21.5	22.9
1,500	872	8,773	91	20.9	22.7
1,600	386	9,136	91	20.6	22.6
All.....	109,116	7,737	90	23.6	24.0

The above table is derived from McDowell's data. The last line shows the average of all records as reported. The data for each breed class have been reduced by a factor such as to give an average for each breed class the same as the average for all records. The breed classes have then been thrown together by size classes, weighting by the number of records, which gives the averages shown in the body of the table.

Turning to the feed cost per hundred-weight of 4 per cent milk, pronounced trends are much less apparent. There is one striking and consistent feature about the feed-cost curves, namely, the feed cost per unit of milk energy is materially lower for grade cows than it is for registered cows. Even in the case of the Jerseys and Holsteins, where the yearly yield for registered stock is

materially higher than that of the grade stock, it is nevertheless the case that the grades produce a unit of milk energy at lower feed cost. The difference amounts to about 10 cents per hundred-weight of 4 per cent milk. This is more than a 10 per cent advantage in feed cost in favor of the grade cows.

ALL BREED CLASSES COMBINED

In order to consider the above matters further the breed classes of table 1 have been combined and the results are given in table 2. The method of combining is outlined in connection with

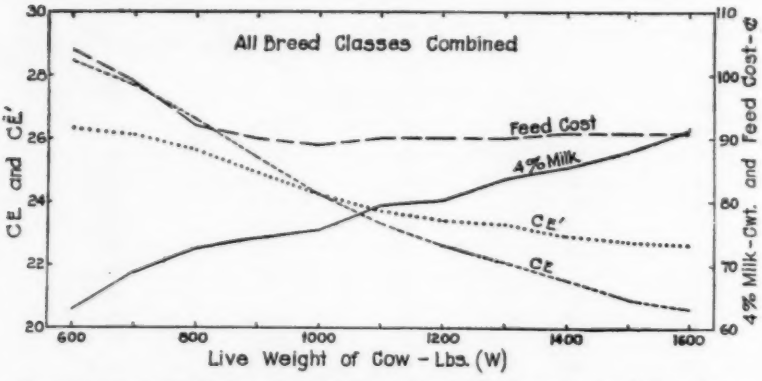

FIG. 2. COMPOSITE RELATION OF SIZE OF COW TO ENERGY YIELD (4 PER CENT MILK), THEORETICAL EFFICIENCY (*CE* AND *CE'*), AND FEED COST PER HUNDRED-WEIGHT OF 4 PER CENT MILK
All breed classes of table 1 combined

table 2, and was intended to regulate for the fact that the various breed classes appear in unequal numbers in the various weight classes. Figure 2 presents the combined data graphically.

The yearly-yield curve, figure 2, becomes quite regular, is substantially linear, and has a slope corresponding approximately to an increase in yearly yield of 250 pounds of 4 per cent milk for each increase of 100 pounds in live weight of cow. It is of interest to note that in advanced registry records there is an increase in 365-day yield of about 500 pounds of 4 per cent milk

for each increase of 100 pounds in live weight of cow (5), (6). Since the average of advanced registry records, ages 5 to 9 years, is also about double that of the present records it seems that the weight-yield relation is similar in the two classes of records, if the level of yield is taken as a base.[3]

A DISCREPANCY IN RESULTS

The feed cost per hundred-weight of 4 per cent milk as shown in figure 2 decreases rapidly over the size-of-cow range from 600 to 800 pounds. From 800 to 1,600 pounds live weight, feed cost per unit of milk is practically constant.

On the other hand, the coefficient-of-efficiency curve, figure 2, shows a continual decrease with size of cow. The feed cost curve is not consistent with the *CE* curve. The coefficient of efficiency represents the percentage of feed energy recovered as milk energy. Feed energy per unit of 4 per cent milk is therefore inversely proportional to *CE*. Ordinarily we should expect feed cost to be directly proportional to feed energy and accordingly the feed cost curve of figure 2 should be the inverse of the *CE* curve. If we plot the reciprocals of *CE*, table 2, the curve is fairly linear. If we further take the average relation of table 2 in which *CE* = 23.6 corresponds to a feed cost of $0.90 and apply it to the

[3] McDowell states: "This study showed that the same relationship in regard to production . . . . as existed among individuals of the same breed did not exist among individuals of different breeds." Table 1 has been arranged to list the breeds in order of increasing average size. Diagonal reading of the yields in table 1 should therefore bear on the point. For greater convenience the following tabulation is repeated from table 1. It shows the effect of successive increases of 100 pounds in live weight on the yield of 4 per cent milk in hundredweights: (a) within the breed, from left to right; (b) breed to breed, from top to bottom of each column.

Grade Jersey	59	64	70	73	75	78
Grade Guernsey	64	68	71	74	77	80
Grade Ayrshire	66	65	69	76	81	81
Grade Holstein	70	74	78	82	86	89

Vertical reading, column by column, does not seem to support the quoted statement. In fact, the breed to breed comparison shows the increase in energy yield for each increase of 100 pounds in live weight to be very similar to that observed for all breed classes combined.

smoothed reciprocals we arrive at a feed cost of $0.75 per hundred-weight of 4 per cent milk at 600 pounds live weight, and $1.05 at 1600 pounds live weight. Each 100 pounds increase in weight should carry with it an increase in feed cost of 3 cents per hundred-weight of 4 per cent milk.

How is the discrepancy in the *CE* and feed cost curves to be accounted for?

Perhaps the *CE* formula is erroneous. If so then our feeding standards for dairy cows are also erroneous. We may consider the possibility that the body maintenance requirements of the cow vary as the 2/3 power of weight instead of being directly proportional to weight. Using the same maintenance requirement at 1,000 pounds live weight we have only to substitute $88.47W^{2/3}$ for $8.847W$ to obtain the new formula, which may be designated *CE'*.

The curve of *CE'* is given in figure 2 for comparison. It does not eliminate the inconsistency with the feed cost curve. It is probable that the actual nutritive requirements lie somewhere between that represented by the two curves, *CE* and *CE'*.

Perhaps the feed cost figures are erroneous, or perhaps feed cost is not proportional to feed energy. The work of Ross et al. (7) bears indirectly on the latter possibility and suggests that the ratio between feed cost and feed energy for marketable feeds would not be affected by size of cow.

It is possible that there may be systematic errors in estimating the feed consumed as between large and small cows, with a tendency to over-charge the small cow and under-charge the large cow. Particularly in the case of pasture this possibility is apparent, since there is a tendency to make the same pasture charge per month per cow regardless of the size of cow. The actual feed energy secured from pasture cannot be accurately estimated but it seems likely that the large cow may profit at the expense of the small cow as a consequence of the system of bookkeeping.

THE POINT OF VIEW

The above treatment of McDowell's data is one way of looking at the matter. McDowell (4) presents the material in quite

a different way, primarily from the point of view of dollar returns per cow per year above feed cost. Of necessity there is a large margin between feed cost and value of product to allow for other costs and a profit. When this margin is multiplied by the amount of product it of course shows favorably to the large cow because of her larger production. It by no means establishes that the large cow is more efficient or even more profitable from a real net profit standpoint.

There can hardly be any doubt that, considering the cow as a machine we want a machine of some size.[4] But it seems to the writer that returns above feed cost may be a misleading way of comparison and lead to an exaggerated conception of the importance of size as one of the factors in the economics of milk production.

SUMMARY

McDowell's (4) published figures of 109,116 yearly records of cows 5 to 9 years of age in dairy herd improvement associations in the United States have been reworked to show, by live weight of cow and breeds: the energy yield in pounds of 4 per cent milk (340 calories); the theoretical coefficient of efficiency, that is, the percentage of feed energy recovered as milk energy; the recorded feed cost in dollars per hundred-weight of 4 per cent milk.

As between grade and registered cows energy yield is substantially equal for the Guernsey and Ayrshire breeds; but materially greater for the registered cows in the case of the Jersey and Holstein breeds.

In all 4 breeds the feed cost per hundred-weight of 4 per cent milk is lower for the grade cows, to the extent of about $0.10. The average feed cost is $0.90 per hundred-weight of 4 per cent milk for all records, the average yearly yield being 7,737 pounds of 4 per cent milk.

[4] For example, the goat is said to be more efficient than the cow, in the sense that there is larger recovery of feed energy as milk energy. Sometime ago while observing the operation of milking in a large goat dairy the writer made a rough estimate that a milker, by hustling, was able to milk at the rate of about 30 pounds per hour. That is, the cost of milking was high due to the small size and output of the individual machine. On the other hand, the advantage of increasing size in this particular may largely disappear when size reaches the neighborhood of, say, 1,000 pounds live weight.

For summary of remaining points all breed classes may be combined.

Energy yield increases with size of cow in a linear manner and at the rate of about 250 pounds of 4 per cent milk per year for each 100 pounds increase in weight. Since this is about half the corresponding figure found in advanced registry records and the average yield also about half, it appears that the weight-yield relation is similar in the two classes of records if level of yield is used as a base.

The theoretical coefficient of efficiency decreases with increasing weight. Interpreted in terms of feed cost this should mean an increase in feed cost per unit of milk with size of cow, amounting to about $0.03 per hundred-weight of 4 per cent milk for each 100 pounds increase in live weight.

The recorded feed cost per hundred-weight of 4 per cent milk decreases with live weight of cow in the range 600 to 800 pounds; and is practically constant in the range 800 to 1,600 pounds.

The cause of the discrepancy between the theoretical efficiency and recorded feed cost cannot be determined from the data at hand. It is suggested that it may be due to systematic errors in the bookkeeping which tend to overcharge the small cow and undercharge the large cow for feed energy actually consumed, particularly in the matter of pasture.

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POSTSCRIPT

Since this paper was turned in for publication an opportunity has occurred in connection with another study, to test in a rough way the postulated relative undercharge to the large cow during the pasture season. The data which have become available are from certain herd books of members of Dairy Herd Improvement Associations in Illinois, during 1927–1929. These books show for each cow a monthly account of milk and fat yield, and cost of feed. The month of calving is given, and there is also a figure for the live weight of the cow for part of the records.

As bearing on the present discussion, selection was made of all those cows with live weight given which calved in December (84 cows), January (116 cows) and February (91 cows). Weight classes were set up by 100 pound intervals (range 600 to 1500 pounds) and the average feed cost per hundredweight of 4 per cent milk determined for the months of April and June. The regressions of feed cost on weight are somewhat irregular but may be fairly represented by straight lines. The method of least squares, weighted by the frequencies, gives the following equations for feed cost in cents per hundred-weight of 4 per cent milk: where W is live weight of cow in hundred-weights.

$$\text{April} = 109.6 - 0.942W$$
$$\text{June} = 90.9 - 2.218W$$

The greater slope of the June curve may be attributed to the use of pasture, and the charge made for it, in that month. There can be little doubt that the system of bookkeeping gives an advantage to the large cow during the pasture season, which results in an apparent lower feed cost of production. The real feed cost, as between cows of different weights, can not be determined from the reported figures of the pasture season. Neither is it at all certain that the winter feed cost figures are entirely free of a defect similar to that prevailing in summer as between cows of different weights. The writer is inclined to the view that the discrepancy in results noted above (page 21) is due to faults in the feed cost figures, rather than to faults in the figures of the nutritive requirements for milk production.

BROMCRESOL GREEN PAPER IN THE MANUFACTURE OF GRAIN-CURD CASEIN*

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In recent work on grain-curd casein, it was found that the end-point of the precipitation,—pH 4.1—may be simply, quickly, and accurately determined by the use of bromcresol green paper. The paper was prepared by dipping S. & S. 589, White Ribbon, filter paper into a 0.4 per cent solution of bromcresol green in 25 per cent ethanol, the solution being adjusted to pH 4.6, potentiometrically measured. The papers were than dried and cut into strips of the usual test paper size. In making a test, a strip of the indicator paper is wet with the whey and stuck on a window pane away from the direct rays of the sun. The color of the paper, viewed by transmitted light, should be compared at once with permanent dry paper standards viewed by reflected light. The protein error is very slight—not more than 0.1 pH unit in whey—and turbidity may be disregarded.

Standards were obtained by comparison of color tests on buffer solutions with Ridgeway's (1) and Maerz and Paul's (2) color standards. Several observers checked each standard. The identifying numbers for each standard selected are given below, together with the corresponding pH value.

	pH 4.0	pH 4.2	pH 4.4	pH 4.6	pH 4.8	pH 5.0
Ridgeway No.....	23′b	25′b	27′b	29′b	31′b	35′b
Maerz and Paul No.....	19-K-1	19-I-1½	19-K-3	18-I-5	19-I-7	19-A-8

An accuracy of 0.1 pH unit has been consistently attained in testing grain-curd casein whey by this quick colorimetric method.

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OBSERVATIONS ON THE HEAT RESISTANCE OF SOME ROPY MILK ORGANISMS*

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While outbreaks of ropiness in market milk have most often occurred with raw supplies, pasteurized milk has occasionally been involved. Outbreaks in the pasteurized product are especially serious because usually a plant selling such milk has a greater number of customers than a plant handling raw milk and also because the appearance of a conspicuous defect in a pasteurized supply tends to discredit the whole pasteurization idea.

The development of ropiness in pasteurized milk has commonly been explained on the basis of contamination from plant equipment following the heating. Recent investigations, however, indicate that old cultures of certain ropy milk organisms may resist pasteurization exposures and, accordingly, it appears that in future outbreaks of ropiness in pasteurized milk two possibilities must be considered, (*a*) contamination following pasteurization and (*b*) causative organisms that are sufficiently heat resistant to survive the pasteurization exposure used.

OBJECT

The work reported herein involved primarily a study of the effect of various exposures at temperatures used in market milk pasteurization on ropy milk organisms secured from pasteurized materials. The object was to determine whether, in the cases studied, the presence of the ropy milk organisms was more likely due to contamination following heating or to their heat resistance. Since the ropy milk organisms secured from the pasteurized materials were all *Bacterium viscosum*, three cultures producing ropiness in milk and belonging to the Escherichia-Aero-

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bacter group were also studied from the standpoint of their heat resistance at milk pasteurization temperatures.

HISTORICAL

Harding and Prucha (1) studied the heat resistance of ropy milk organisms from a number of outbreaks of ropy milk. The tests were carried out in Sternberg bulbs and the materials heated included bouillon suspensions of twenty-four-hour agar slope cultures, eighteen-hour milk cultures that were ropy and sterile milk to which growth from twenty-four-hour agar slope cultures had been transferred by means of water. The organisms used were largely *Bacterium viscosum* but representatives of the *Bacterium aerogenes* group were also included. These investigators concluded that, ". . . . it seems clear that proper pasteurization of milk at 140° to 145° for thirty minutes should destroy any ropy milk organisms which may be in the milk."

In a study of the comparative resistance of young and mature bacterial cells to various factors, Sherman and Albus (2) noted that the young cells were more rapidly destroyed by heating to relatively low temperatures than were the older cells.

Wolff (3) investigated the influence of the age of milk on the results of pasteurization and reported data showing the effect of heating to 62° to 63°C. for 30 minutes on the bacterial content of milk when fresh and again after a holding period; both gelatin and agar plates were used. The milk pasteurized after holding regularly contained more organisms than the milk pasteurized when fresh and in some, but not all, of the instances the percentage of the organisms which survived pasteurization was greater with the milk that had been held than with the fresh milk.

Stark and Stark (4) studied the thermal death rates of young and mature cells of *Streptococcus fecalis* and of "two species of capsulated bacteria" isolated from ropy milk and found the young cells were markedly less resistant than the mature cells. These investigators point out that, "Of practical interest is the fact that the thermal death rates of the young and old cells are so different that such commercially important organisms as the 'ropy milk' bacteria may survive pasteurization or be entirely eliminated, depending upon the physiological age of the cells."

Sherman, Stark and Stark (5) found that milk kept at temperatures (e.g., 15.5°C.) permitting bacterial growth showed a greater percentage destruction of bacteria during pasteurization than similar milk when fresh or after holding at temperatures (e.g., 4.4°C.) too low to allow multiplication of the bacteria involved. In their summary they state that, "It has been established that some important milk bacteria (e.g., 'ropy milk' types) may be entirely eliminated from milk by pasteurization when the cells are in a young and growing condition, while the old cells of the same organisms are able to withstand the process."

Scales (6) investigated an outbreak of ropy milk and found that the cells present in milk held at low temperatures were more resistant to pasteurization than cells that had been allowed to develop for a short time. He pointed out that the efficiency of the pasteurizing process was increased by holding the milk at 58°F. for 45 minutes before the milk was pasteurized.

METHODS

Six heating trials were carried out, each involving a given temperature for various periods. Except in trials 1 and 6, organisms from more than one source were employed so that generally a trial included a considerable number of individual exposures.

The cultures of ropy milk organisms used were grown in milk, in diluted milk (1 part milk to 9 parts distilled water) or on beef infusion agar slopes for varying lengths of time. The diluted milk was intended to approximate the growth conditions encountered in equipment left with considerable quantities of milk solids and water in it.

All the heat exposures were made in milk, using a milk culture direct (trial 1 only) or adding material from a culture in diluted milk or on an agar slope to the milk shortly before the heating. Usually sterilized milk was employed but trial 2 was carried out with aseptically drawn milk since it was thought the physical effect of sterilization might be a factor in the heat resistance of an organism in milk.

The cultures were exposed to heat in thin walled test tubes (about 8 mm. inside diameter and about 75 mm. long) or in

capillary pipettes. When the test tubes were used, 2 cc. of the milk containing one of the organisms were sealed in each tube in trials 1 and 2, while 1 cc. was used per tube in the other trials; the sealed tubes were from 45 to 55 mm. long. With the capillary pipettes an amount of the milk estimated at from one-fortieth to one-twentieth of a cubic centimeter was drawn into a pipette and the lower end then sealed.

The heating was carried out in a large water bath supported on tripods; the temperature could be kept constant and uniform within 0.2°C. of that desired by the proper regulation of the flames beneath and occasionally stirring. When the heating involved only sealed tubes, these were tied together in bunches and each bunch carried to the desired depth in the water by means of a weight. The pipettes were held in the water by fitting them into slits sawed in a board that could be placed across the top of the water bath; the water was deep enough so that the surface was several inches above the top of the milk in the pipettes. When both a pipette and a sealed tube were to be used with a given exposure, the tube was attached to the lower end of the pipette by means of a rubber band and the pipette then held in the usual way.

In the later trials (trials 4, 5 and 6) the sealed tubes or capillary pipettes containing the milk to be heated were plunged into the water bath within a very few minutes of the completion of their preparation. The procedure in the earlier trials varied; in trials 1 and 2 the tubes used were sealed and then held in ice water until all were ready to be heated, while in trial 3 the sealed tubes were held at room temperature until they were all prepared.

As soon as a heating exposure was completed, the sealed tube or pipette used was plunged into cold water so that the temperature of the milk containing the organisms would fall rapidly.

The survival or destruction of an organism under study was determined by inoculating the heated milk into sterile litmus milk, holding at room temperature and observing for growth after various periods up to one week. The sealed tubes were opened by scratching with a file and then touching with a hot glass rod, and 0.5 cc. of the heated milk was employed. The

lower end of each capillary pipette was held for a few minutes in a 5 per cent solution of $HgCl_2$ in 95 per cent alcohol and, after wiping with sterile cotton, the tip of the pipette was broken off and the milk blown out as completely as possible. In the tests with aseptic milk growth of organisms other than the ropy milk types added was occasionally noted after comparatively long holding periods. The organisms involved were present in the aseptically drawn milk and were so distinctly different from the types being studied that there was no uncertainty as to the results.

In trials 1 and 2 counts were made on some of the milk ready for heating in order to secure an idea of the number of organisms present per cubic centimeter. The results are as follows:

Trial 1, culture A, 7 hour milk culture 7,300,000 per cubic centimeter, 55 hour milk culture 1,505,000,000 per cubic centimeter, and 103 hour milk culture 1,060,000,000 per cubic centimeter; trial 2, culture A, milk plus 7 hour diluted milk culture 267,000 per cubic centimeter, milk plus 79 hour diluted milk culture 12,100,000 per cubic centimeter and milk plus 79 hour agar slope culture 61,500,000 per cubic centimeter; trial 2, culture B, milk plus 7 hour diluted milk culture 254,000 per cubic centimeter, milk plus 79 hour diluted milk culture 6,325,000 per cubic centimeter and milk plus 79 hour agar slope culture 79,000,000 per cubic centimeter.

RESULTS OBTAINED

Series A

The ropy milk organism used in series A was isolated from a sample of pasteurized milk sent to the Iowa Agricultural Experiment Station from a neighboring state by a plant having trouble with ropiness in their pasteurized product. The milk was shipped without ice and was sour on arrival. Cultures causing pronounced ropiness in milk were easily isolated by direct plating and also by inoculating some of the milk into sterile milk, holding at about 7°C. until ropiness developed and then plating. Beef infusion agar and an incubation of two days at room temperature were used. All the cultures secured were of one type which was identified as *Bacterium viscosum*.

TABLE 1

Heat resistance of Bacterium viscosum from ropy pasteurized milk

	CULTURES USED	TEMPERATURE OF HEATING	PERIODS OF HEATING	SURVIVALS	
				Sealed tubes	Capillary pipettes
		°C.	minutes		
Trial 1	A and B 7 hours in diluted milk	62.8	10, 20, 30	None	
	A and B 55 hours in diluted milk	62.8	10, 20, 30	None	
	A and B 103 hours in diluted milk	62.8	10, 20, 30	None	
Trial 2	A and B 7 hours in diluted milk	62.8	10, 20, 30	None	
	A anb B 79 hours in diluted milk	62.8	10, 20, 30	None	
	A and B 79 hours on agar	62.8	10, 20, 30	None	
Trial 3	A and B 6 hours in diluted milk	62.8	10, 20, 30	None	
	A and B 103 hours in diluted milk	62.8	10, 20, 30	None	
	A and B 103 hours on agar	62.8	10, 20, 30	None	
Trial 4	A and B 6 hours in diluted milk	62.8	5, 10	None	None
	A 30 hours in diluted milk	62.8	5, 10	None	None
	A and B 54 hours in diluted milk	62.8	5, 10	None	None
	A and B 6 hours on agar	62.8	5, 10	None	None
	A 30 hours on agar	62.8	5, 10	None	None
	A and B 54 hours on agar	62.8	5, 10	None	None
Trial 5	A and B 6 hours in diluted milk	61.1	3, 5, 10	None	None
	A and B 54 hours in diluted milk	61.1	3, 5, 10	None	None
	A and B 6 hours on agar	61.1	3, 5, 10	None	None
	A and B 54 hours on agar	61.1	3, 5, 10	None	None

The heat resistance of the organism at temperatures commonly employed for pasteurization of market milk was studied using two of the cultures isolated, cultures A and B. The results obtained are presented in table 1. The data show that the organism from cultures of various ages in diluted milk or on agar slopes failed to survive any of the exposures, which varied from 3 minutes at 61.1°C. to 30 minutes at 62.8°C. The findings suggest that the organism causing the ropiness did not resist the heating but gained entrance to the milk after pasteurization.

TABLE 2

Heat resistance of Bacterium viscosum from pasteurized cream butter

	CULTURES USED		TEMPERATURE OF HEATING	PERIODS OF HEATING	SURVIVALS	
					Sealed tubes	Capillary pipettes
			°C.	minutes		
Trial 4	C and D	6 hours in diluted milk	62.8	5, 10	None	None
	C	30 hours in diluted milk	62.8	5, 10	None	None
	C and D	54 hours in diluted milk	62.8	5, 10	None	None
	C and D	6 hours on agar	62.8	5, 10	None	None
	C and D	54 hours on agar	62.8	5, 10	None	None
Trial 5	C	6 hours in diluted milk	61.1	3, 5, 10	None	None
	C	54 hours in diluted milk	61.1	3, 5, 10	None	None
	C	6 hours on agar	61.1	3, 5, 10	None	None
	C	54 hours on agar	61.1	3, 5, 10	None	None

Series B

An unusual defect in a sample of butter sent to the Iowa Butter Control Laboratory furnished the ropy milk organism used in series B. On arrival the butter showed a number of rather large moisture droplets at the surface, each of which was so distinctly ropy that filaments an inch or more in length could be drawn out with a needle. Since the butter was made from pasteurized cream, it presented the same problem, from the standpoint of the source of the ropy milk organism, as does ropy pasteurized milk. Cultures which produced a pronounced ropiness in milk were secured by plating one of the moisture droplets and also the

butter after the moisture had been redistributed as thoroughly as possible, using beef infusion agar and an incubation of two days at room temperature; these were all of one type which was identified as *Bacterium viscosum*.

Two of the cultures isolated, cultures C and D, were used in heat resistance tests at the pasteurizing temperatures usually employed with market milk; the data obtained are given in table 2. Although the organism was taken from cultures of various ages in diluted milk or on agar slopes, it failed to survive any of the exposures which ranged from 3 minutes at 61.1°C. to 10

TABLE 3

Heat resistance of Bacterium viscosum from ropy whey secured from pasteurized milk

	CULTURES USED	TEMPERATURE OF HEATING	PERIODS OF HEATING	SURVIVALS	
				Sealed tubes	Capillary pipettes
		°C.	minutes	minutes	
Trial 6	E 7 hours in diluted milk	61.1	1, 3, 5, 10, 20	1	None
	E 54 hours in diluted milk	61.1	1, 3, 5, 10, 20	1	None
	E 7 hours on agar	61.1	1, 3, 5, 10, 20	1	None
	E 54 hours on agar	61.1	1, 3, 5, 10, 20	1	None

minutes at 62.8°C. The results accordingly indicate that the organism did not survive the heating of the cream but gained entrance to the cream or butter following the pasteurization.

Series C

A ropy milk organism secured from ropy whey was used in series C. The whey came from a vat of pasteurized whole milk set for the manufacture of cream cheese. After an overnight holding period the small amount of whey at the surface of the material showed a number of slightly brown areas, each approximately round and 2 to 3 inches in diameter. The whey in these areas was very ropy, while the remaining whey was not. Plates poured with the ropy whey, using beef infusion agar and an incubation of two days at room temperature, developed colonies that rapidly produced ropiness in milk into which they were

inoculated. The ropy milk cultures secured appeared to be identical and were found to be *Bacterium viscosum*.

One of the isolated cultures, culture E, was used in heat resistance tests at 61.1°C. The results obtained, which are presented in table 3, show that in sealed tubes the organism survived a one minute heating period but failed to survive the longer periods (3, 5, 10 or 20 minutes), while in capillary pipettes it failed to survive the one minute exposure. It thus appears that the organism did not live through the pasteurization of the whole milk used but gained entrance following the heating.

Series D

The Escherichia-Aerobacter group has been responsible for outbreaks of ropiness in both raw and pasteurized milk and since all of the ropy milk organisms secured from the materials with which pasteurization had been used were *Bacterium viscosum*, 3 cultures producing ropiness in milk and belonging to the Escherichia-Aerobacter group were studied as to their heat resistance at common milk pasteurization temperatures. All 3 cultures were *Aerobacter aerogenes*. They had been in the stock cultures for several years and were secured in the picking of colonies from plates for the purpose of studying the general types of organisms present; the principal reason for keeping them was their ability to produce ropiness in milk. Culture F came from sour cream, culture G from a cow's mouth and culture H from sour cream.

The results obtained on the three *Aerobacter aerogenes* organisms, using various exposures at temperatures commonly employed for market milk pasteurization, are given in table 4. With culture F organisms from a young diluted milk culture failed to survive 62.8°C. for 10 minutes, while those from an old culture in diluted milk or on an agar slope survived this temperature for 10 minutes but not for 20 minutes. Culture G failed to survive any of the exposures used; in trial 3 organisms from a young culture in diluted milk or from old cultures in diluted milk or on an agar slope were destroyed at 62.8°C. for 10 minutes, which was the shortest period used, and in trial 5 organisms from young

TABLE 4

Heat resistance of Aerobacter aerogenes from various sources

	TYPE OF CULTURES USED	TEMPERATURE OF HEATING	PERIODS OF HEATING	SURVIVALS	
				Sealed tubes	Capillary pipettes
Culture F					
		°C.	*minutes*	*minutes*	
Trial 3	6 hours in diluted milk	62.8	10, 20	None	
	103 hours in diluted milk	62.8	10, 20	10	
	103 hours on agar	62.8	10, 20	10	
Culture G					
Trial 3	6 hours in diluted milk	62.8	10, 20, 30	None	None
	103 hours in diluted milk	62.8	10, 20, 30	None	None
	103 hours on agar	62.8	10, 20, 30	None	None
Trial 5	6 hours in diluted milk	61.1	3, 5, 10	None	None
	54 hours in diluted milk	61.1	3, 5, 10	None	None
	6 hours on agar	61.1	3, 5, 10	None	None
	54 hours on agar	61.1	3, 5, 10	None	None
Culture H					
Trial 2	7 hours in diluted milk	62.8	10, 20, 30	None	
	79 hours in diluted milk	62.8	10, 20, 30	10	
	79 hours on agar	62.8	10, 20, 30	10, 20	
Trial 3	6 hours in diluted milk	62.8	10, 20, 30	None	
	103 hours in diluted milk	62.8	10, 20, 30	10	
	103 hours on agar	62.8	10, 20, 30	10	
Trial 5	6 hours in diluted milk	61.1	10, 15, 20, 30	None	None
	54 hours in diluted milk	61.1	10, 15, 20, 30	10	None
	6 hours on agar	61.1	10, 15, 20, 30	None	None
	54 hours on agar	61.1	10, 15, 20, 30	10, 15, 20	10, 15, 20

or old cultures in diluted milk or on agar slopes were destroyed at 61.1°C. in 3 minutes. Culture H was the most resistant to heat of any of the cultures studied. Organisms from old cultures in diluted milk or on agar slopes regularly resisted 61.1°C. or 62.8°C. for 10 minutes and in some instances for 20 minutes, while organisms from the young cultures failed to survive these temperatures for 10 minutes. In trials 2 and 5 organisms from the old cultures on agar slopes were more resistant than those from the old cultures in diluted milk but this variation was not evident in trial 3 with the exposures used.

DISCUSSION OF RESULTS

The ropy milk organisms secured from pasteurized milk and products made from pasteurized milk or cream showed very little resistance to heat at the temperatures commonly used for milk pasteurization, although cultures of various ages grown under different conditions were employed. This indicates that some of the outbreaks of ropiness are due to contamination following heating and thus confirms the work of Harding and Prucha (1). In connection with the work of Stark and Stark (4) and Scales (6), it leads to the conclusion that ropiness in pasteurized dairy products may be due to either contamination following the pasteurization or causative organisms sufficiently heat resistant to survive the pasteurization exposure used.

The outbreak of ropiness in pasteurized milk which provided the organisms used in Series A was handled as follows: As soon as the ropiness was noticed a sample was taken from the milk of each patron and held over night. The next morning 27 of the 180 samples showed evidence of ropiness and the milk from the farms represented by the 27 samples was rejected. At the same time the rinsing of all the plant equipment, night and morning, with a solution of a chlorine compound was begun. With this procedure the ropiness in the pasteurized milk disappeared immediately. The excluded supplies were gradually taken back following sterilization of equipment at the farms and the submitting of samples that failed to develop ropiness. The plant employee who studied the outbreak concluded that the ropy milk organisms were spread from the plant to the farms.

The three *Aerobacter aerogenes* cultures studied, which produced ropiness in milk although not coming from ropy dairy products, varied in their heat resistance and two of the cultures were definitely more resistant than the *Bacterium viscosum* cultures. The results secured with these two cultures confirmed the observations of various investigators, especially those of Sherman and Albus (2) and of Stark and Stark (4), that cells from young cultures are less resistant than those from old cultures. In two of the three trials with one *Aerobacter aerogenes* culture the cells from an old agar slope culture were more resistant than those from an old diluted milk culture; it should be noted, however, that according to the counts given under "Methods" the former were present in larger numbers per cubic centimeter of the material heated than the latter.

SUMMARY

Ropy milk organisms were isolated from (*a*) an outbreak of ropiness in pasteurized milk, (*b*) butter which showed ropy moisture droplets at the surface and which was made from pasteurized cream and (*c*) ropy whey obtained from pasteurized milk set for the manufacture of soft cheese. All proved to be *Bacterium viscosum*. In heat resistance trials the organisms failed to survive 61.1°C. for 3 minutes; these trials involved primarily the exposure of organisms added to milk after growing for varying periods in diluted milk or on agar slopes, although in trial 1 young and old milk cultures were used direct.

Three cultures of *Aerobacter aerogenes* that produced ropiness in milk were studied for their heat resistance and found to vary greatly in this character. With culture F. organisms from an old diluted milk or agar slope culture survived 62.8°C. for 10 minutes but those from a young milk culture did not; with culture G organisms from either a young or old diluted milk or agar slope culture failed to survive 61.1°C. for 3 minutes; with culture H organisms from an old diluted milk or agar slope culture regularly resisted 62.8°C. for 10 minutes and in some instances for 20 minutes although organisms from young cultures failed to resist these exposures. In two trials with culture H organisms from

old cultures on agar slopes were more resistant than those in diluted milk.

The results indicate that in some instances ropiness in pasteurized milk or products made from it is due to contamination following the heating and that this possibility should be considered along with heat resistant causative organisms.

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VARIATIONS IN THE COAGULATION AND PROTEOLYSIS OF MILK BY STREPTOCOCCUS LACTIS*

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The variations occurring among the organisms that are included in a species and also among the cultures secured by plating what is believed to be a pure culture greatly complicate the classificaton of the bacteria. Variety and type designations have been introduced with some species to point out differences that appear to be important without over-emphasizing them, but the significance to be attached to variations is often difficult to determine.

The streptococci found in dairy products present a classification problem that is of special importance because of their extensive development in these materials, the variety of changes which they produce and the lack of well defined boundaries between the types. Even with the streptococci that produce considerable quantities of lactic acid in milk, variations in the type and rate of the changes produced, as well as in the morphology, make the development of a logical scheme of grouping very difficult.

When a supposedly pure culture of *Streptococcus lactis* is plated and colonies picked into litmus milk the cultures secured sometimes show a wide variation in their rates of coagulation. A similar variation may occur among the cultures other than the citrate fermenters that are secured when a butter culture developed by combining a *S. lactis* culture with a citrate fermenter is plated and colonies picked into litmus milk. This variation among organisms which presumably came from the same original culture but which differ so widely in the rates at which they produce changes in milk brings up the question of the relationship of these types.

OBJECT

The work herein reported was carried out to determine to what extent *S. lactis* cultures varying widely in their coagulation rates

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could be obtained from plates poured with purified strains of *S. lactis*. The rates at which milk is coagulated may be a deceptive basis on which to compare cultures because a slight difference in the amount of acid formed can result in one culture coagulating milk and another not, so the study of an additional change which, in general, varies in accordance with variations in the rate of coagulation seemed advisable. The correlation between rapid coagulation and pronounced proteolysis of milk that occurs with *S. lactis* cultures appeared to meet the requirements and, accordingly, the proteolyzing power of many of the cultures secured was investigated.

HISTORICAL

The results of various investigations have shown that at least certain strains of *S. lactis* are capable of bringing about protein decomposition in milk; these have recently been reviewed by Hammer and Patil (1).

Anderegg and Hammer (2) isolated a number of *S. lactis* cultures from various dairy products and studied their proteolytic activity in milk, with and without $CaCO_3$, using as a rule fourteen days incubation at room temperature. In general the cultures coagulating milk rapidly caused a definite proteolysis while the slowly coagulating cultures brought about little, if any, protein decomposition. Hammer and Patil (1) reported that a considerable number of *S. lactis* cultures investigated fell into two types, proteolytic and non-proteolytic. The first rapidly coagulated milk at room temperature, while the second showed considerable variation in the coagulation rate but never coagulated as rapidly at this temperature as the proteolytic type. The general correlation between the proteolytic activity and coagulation rate of *S. lactis* did not hold at 30° or 37°C. since at these temperatures some of the non-proteolytic strains coagulated milk about as rapidly as the proteolytic ones.

GENERAL PROCEDURE

The procedure used with each *S. lactis* culture consisted of plating on whey agar, picking contiguous colonies (about twenty-five)

into litmus milk after there was a good development at room temperature and comparing at 21.1°C. the coagulation rates of the cultures secured directly from the plate. A pronounced difference in the rates of coagulation was checked by making transfers with a loop to tubes of litmus milk and noting the periods required for coagulation at 21.1°C. In most of the instances in which a definite difference in the coagulation rates was established, representatives of both the slowly and rapidly coagulating cultures were studied for their ability to proteolyze milk.

The original cultures were secured from various sources and were purified by plating on whey agar and picking colonies into litmus milk three times before beginning their study. Single cell isolations were not used because the primary object of the work was to determine the relationship of organisms isolated from dairy products by the plate method.

Since previous investigations at the Iowa Agricultural Experiment Station had shown that an increase in soluble nitrogen is regularly accompanied by an increase in amino nitrogen, the proteolyzing powers of a culture were studied by determining the change produced in the amino nitrogen content of milk in seven days at room temperature; $CaCO_3$ was added to the milk (before sterilization) and frequently distributed (by shaking) during the growth period since the neutralization of some of the acid in this way permits a greater growth of the organism and more extensive proteolysis by a proteolyzing type. The amino nitrogen was determined by the Van Slyke method using 10 cc. of a filtrate from the milk for a determination and limiting the foaming with 1 cc. of butyl alcohol. The amino nitrogen values reported represent the difference in milligrams between the amino nitrogen content of 10 cc. of filtrate from milk in which an organism had grown and that of 10 cc. of filtrate from an uninoculated portion of the same milk. The filtrate from the milk was obtained by the method already described by Anderegg and Hammer (2) and also by Hammer and Patil (1).

RESULTS OBTAINED

The work was divided into two parts. In Part I the original cultures were carried through litmus milk transfers and whenever

plates were to be poured an unselected transfer was used. In Part II the original cultures were run through a series of platings, a rapidly coagulating culture secured from one plating being used to pour the next set of plates. The principal difference between the procedures in Part I and Part II is that in Part I after the original purification of the cultures there was no additional purification, while in Part II there was a continual purification throughout the series of platings, each culture plated having been through one more plating than the culture preceding it.

TABLE 1

Designations, sources and types of the cultures used in Part I

CULTURE	SOURCE	TYPE
A1 and A2	Sour cream	Typical *S. lactis*
B1 and B2	Sour cream	*S. lactis var. maltigenes*
C1 and C2	Stock culture	Typical *S. lactis*
F1 and F2	Fresh cow feces	Typical *S. lactis*
H1 and H2	Commercial butter culture	Typical *S. lactis*
K1 and K2	Milk	*S. lactis var. maltigenes*
K3	Stock culture	*S. lactis var. maltigenes*
M1 and M2	Milk	*S. lactis var. hollandicus*
O1 and O2	Milk	Typical *S. lactis*

Part I

In Part I 17 cultures were used; the designations, sources and types of these are given in table 1. The data show that the organisms studied came from various sources and included typical *S. lactis*, *S. lactis var. maltigenes* and *S. lactis var. hollandicus*.

With each organism 6 sets of whey agar plates were poured, using the original culture or a transfer (two to four days old) coming from the series of transfers made from it. After incubating at 21.1°C. until there was a good development, a plate showing a satisfactory distribution of colonies was selected and about twenty-five contiguous colonies picked into litmus milk.

When one or more cultures that appeared to be slower in their rates of coagulation than the original culture were secured, this slow coagulation was checked by a series of loop transfers to litmus milk using rapidly coagulating cultures from the same plate

for comparison. Some of the cultures that coagulated slowly when first picked from the plates proved to be as rapid as any of the cultures, the slow original coagulation undoubtedly being due to the inoculation of a very small number of cells or some such factor, but most of the cultures coagulating slowly when first picked continued to show a delayed coagulation in subsequent inoculations.

TABLE 2

Numbers of slowly coagulating cultures obtained when about twenty-five colonies were picked from a plate—Part I

CULTURE	PLATING					
	1	2	3	4	5	6
A1	0	0	0	1	1	0
A2	0	0	0	0	0	0
B1	0	1	2	0	2	1
B2	0	1	2	0	2	1
C1	0	0	0	2	1	0
C2	0	0	0	0	1	0
F1	0	0	1	0	0	0
F2	0	0	0	0	2	0
H1	0	0	0	0	0	0
H2	0	0	0	0	0	0
K1	0	0	0	0	1	0
K2	0	1	1	1	1	0
K3	0	0	0	0	0	0
M1	0	2	2	0	2	1
M2	0	0	0	0	0	1
O1	1	1	0	2	3	1
O2	2	1	0	2	2	1

The numbers of slowly coagulating cultures that were secured from the various platings are summarized in table 2. The results show that slowly coagulating cultures were never secured in all six of the platings of an organism; they were secured in five of the platings with two organisms (12 per cent), in four of the platings with four organisms (24 per cent), in two of the platings with two organisms (12 per cent), in one of the platings with five organisms (29 per cent) and in none of the platings with four organisms. The numbers of slowly coagulating cultures secured per 25 colonies

picked from a plate varied from none to three (from 0 to 12 per cent); the total numbers secured from the six platings of one organism ranged from none to eight (from 0 to 5 per cent); of the approximately 2550 cultures picked from the plates, fifty (1.96 per cent) were slow coagulators.

From each plating which yielded cultures differing definitely in their rates of coagulation, a slowly coagulating culture and a

TABLE 3

Proteolysis by rapidly and by slowly coagulating cultures—Part I

CULTURE	PLATING	INCREASE (OVER CHECK) AMINO N PER 10 CC. FILTRATE		CULTURE	PLATING	INCREASE (OVER CHECK) AMINO N PER 10 CC. FILTRATE	
		Rapid	Slow			Rapid	Slow
		mgm.	*mgm.*			*mgm.*	*mgm.*
A1	4	0.132	0.055	M1	2	2.007	0.145
A1	5	0.269	0.140	M1	3	1.512	0.191
B1	2	0.746	0.053	M1	5	1.456	−0.052
B1	3	0.631	0.301	M1	6	2.257	0.121
B1	5	0.798	−0.025	M2	6	0.767	0.121
B1	6	0.767	−0.007	O1	1	0.936	0.063
B2	2	0.807	0.059	O1	2	1.227	0.006
B2	3	0.989	0.163	O1	4	0.817	0.188
B2	5	−0.661	0.052	O1	5	1.088	0.026
B2	6	1.080	0.067	O1	6	0.824	0.027
C1	4	0.837	0.077	O2	1	0.626	0.081
C1	5	0.853	0.086	O2	2	0.760	−0.081
C2	5	0.054	0.027	O2	4	1.003	0.106
F1	3	1.508	0.043	O2	5	0.557	0.053
F2	5	0.038	0.091	O2	6	0.974	0.000
K1	5	1.357	0.074				
K2	2	0.882	0.110				
K2	3	1.045	0.270				
K2	4	1.061	0.021				
K2	5	1.220	0.074				

rapidly coagulating one were selected and studied for their proteolyzing power. The results obtained are given in table 3. Of the 35 pairs of cultures studied, in all but four the rapidly coagulating culture definitely gave more proteolysis than the slowly coagulating one. With these four the rapidly coagulating cultures failed to produce as extensive a proteolysis as that produced by the rapidly coagulating cultures in the other 33 comparisons.

A number of pairs of cultures (each pair representing a plate) with which there was a definite difference in their rates of coagulation at 21.1°C. were compared at 30° and 37°C. but at these temperatures there was no difference between the cultures constituting a pair. This is in agreement with the observations reported by Hammer and Patil (1).

Part II

Four cultures were used in Part II, all of which were isolated from sour cream. H1 and H2 were *S. lactis var. maltigenes*, while H3 and H4 were typical *S. lactis*.

Each of the organisms was run through a series of platings on whey agar at 21.1°C., using a rapidly coagulating culture (two to four days old) from one plating as inoculating material for the next. After a satisfactory development had taken place with the plates in each set, a plate showing a good colony distribution was selected and about twenty-five contiguous colonies picked into litmus milk and incubated at 21.1°C. From some of the plates one or more cultures that were comparatively slow in coagulating milk were secured. The relative coagulation rate of each of these was checked against a rapidly coagulating culture from the same plate by making loop transfers to litmus milk. As in Part I a few of the cultures originally selected as slow proved to be rapid, while the others continued to coagulate milk slowly during a series of transfers.

Table 4 shows the numbers of cultures coagulating milk slowly that were secured from each plating of each organism. Such cultures were secured from nine (31 per cent) of the twenty-nine platings of H1, from seven (27 per cent) of the twenty-six platings of H2, from three (10 per cent) of the thirty-one platings of H3 and from eight (26 per cent) of the thirty-one platings of H4. The numbers of slowly coagulating cultures secured per twenty-five colonies picked from a plate varied from none to nine (0 to 36 per cent); of the approximately 2925 cultures picked from the plates, forty-seven (1.61 per cent) were slow coagulators.

In all but one of the platings from which slowly coagulating cultures were secured, one (occasionally more) of these cultures

was compared as to proteolyzing ability with a rapidly coagulating culture from the same plate. The results are given in table 5.

TABLE 4

Numbers of slowly coagulating cultures obtained when about twenty-five colonies were picked from each plate in the regular series—Part II

PLATING	CULTURE			
	H1	H2	H3	H4
1	1	0	0	0
2	0	1	0	0
3	1	2	0	0
4	1	1	0	0
5	0	0	0	0
6	0	0	1	0
7	3	1	0	0
8	0	0	0	2
9	0	1	0	1
10	0	0	0	1
11	1	0	0	1
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
17	0	0	1	0
18	1	0	0	0
19	0	1	0	0
20	0	0	0	0
21	0	1	0	0
22	1	0	0	0
23	0	0	0	0
24	1	0	0	0
25	0	0	0	0
26	0	0	0	0
27	1		0	4
28	0		1	1
29	0		0	0
30			0	6
31			0	9

The rapidly coagulating culture in a pair always gave more proteolysis than the slowly coagulating one and usually the difference was marked.

In an attempt to increase the incidence of the slowly coagulating cultures secured from a plate, additional platings were made using a young uncurdled culture in some instances and an old culture (up to six days) in others. The general procedure followed was that already outlined. Both the young and old cultures yielded approximately the same number of slow coagulators as the cultures plated in the regular series. Some of the slowly coagulating cultures obtained from either a young or an old culture were studied for their proteolyzing power in milk and each gave much

TABLE 5

Proteolysis by rapidly and by slowly coagulating cultures—Part II

PLATING	H1		PLATING	H2		PLATING	H3		PLATING	H4	
	INCREASE (OVER CHECK) AMINO N PER 10 CC. FILTRATE			INCREASE (OVER CHECK) AMINO N PER 10 CC. FILTRATE			INCREASE (OVER CHECK) AMINO N PER 10 CC. FILTRATE			INCREASE (OVER CHECK) AMINO N PER 10 CC. FILTRATE	
	Rapid	Slow		Rapid	Slow		Rapid	Slow		Rapid	Slow
	mgm.	*mgm.*		*mgm.*	*mgm.*		*mgm.*	*mgm.*		*mgm.*	*mgm.*
1	1.447	0.407	2	1.043	0.165	6	1.637	0.691	8	1.632	0.182
3	1.047	0.386	3	0.967	0.030	17	0.879	0.000	9	1.548	0.116
4	1.060	−0.123	4	0.791	0.123	28	0.997	0.332	10	1.987	0.111
7	0.772	0.278	7	0.993	0.621				11	1.561	0.061
11	1.561	0.227	9	1.159	0.992				27	1.319	0.000
18	1.114	0.663	19	0.987	0.000				28	1.486	−0.056
22	1.510	0.691	21	1.129	0.797				30	1.321	0.182
27	0.932	0.274							31	1.340	0.210

less proteolysis than a rapidly coagulating culture from the same plate.

A number of the slowly coagulating cultures secured were plated on whey agar and after a good colony development had occurred at 21.1°C. twenty-five contiguous colonies were picked from one plate in each set and incubated at the same temperature. The cultures obtained in this way all coagulated milk slowly. There was some variation in the coagulation rates of the colonies from a plate but this was slight and not comparable with the variation often noted among the colonies from a plate poured with a rapidly coagulating culture.

SUMMARY AND CONSIDERATION OF RESULTS

The results presented show that certain pure cultures of *S. lactis* rapidly coagulating milk can be split into rapidly and slowly coagulating strains and that, in general, the former produce considerable proteolysis in milk, while the latter bring about little or no proteolysis. The general correlation between rapid coagulation and pronounced proteolysis with *S. lactis* cultures that has been noted with isolations from various sources thus appears to persist when variations occur in the rates of coagulation of cultures picked from a plate poured with a pure culture. In Part I the percentage of the approximately 2550 colonies picked that were slow coagulators was 1.96, while in Part II the percentage of the approximately 2925 colonies was 1.61. These percentages agree quite closely with the percentage (2.4) of non-ropy cultures of *S. lactis* secured by Hammer (3) when 1288 colonies were picked from plates poured with ropy cultures.

In the trials carried out the cultures secured by plating a slowly coagulating culture and picking colonies into litmus milk were all slow in their coagulation rates. It thus appears to be much more difficult to secure rapidly coagulating cultures from slow ones than to secure slowly coagulating cultures from rapid ones.

The variations in the rates of coagulation and the extents of proteolysis among the cultures secured when plating what would ordinarily be regarded as a pure culture of *S. lactis* make these characteristics of little value in the classification of this group. They also indicate that pronounced differences in the coagulation rates of cultures secured from a plate poured with a dairy product do not *necessarily* indicate a difference in species.

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VARIATIONS IN THE BUFFER VALUE OF HERD MILK*

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Interest has been directed recently to the importance of the buffer value of milk, particularly in regard to dairy manufacturing and nutritional studies. The buffer intensity of milk is a function of its acid-base equilibrium, and is especially significant in relation to the casein and phosphate systems. Whittier (1), and Holm and Webb (2) of these laboratories have already pointed out the importance of the buffer index of milk; the former has investigated its relation to various milk constituents, and the latter have indicated its significance in nutrition, especially with reference to the composition of infant foods.

Many industries are fortunate in the fact that their raw materials are very uniform in quality or may be easily controlled. However, the basic material of the dairy industry is a biological fluid, which varies markedly from time to time and from place to place, the variations being due to many factors.

Considerable information has been published concerning the variation in the constituents of milk, but little knowledge is available concerning variations in the acid-base equilibrium of milk which have an important bearing on certain processes in the manufacture of casein, cheese and condensed milk products. It was thought, therefore, that these considerations warranted the presentation of some studies which indicate the variations which may occur in the buffer capacity of milk.

THEORETICAL

The theoretical aspects of buffering, and the calculation of the buffer index, will be treated only briefly here, inasmuch as Van Slyke (3) has discussed the subject quite fully. Additional in-

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formation with particular reference to milk will be found in the work of Clark (4), Buchanan and Peterson (5), and Whittier (1).

The buffer value of a solution may be expressed as its power to resist change in pH upon the addition or loss of acid or alkali. Experimentally, standard acid or alkali in measured amounts is added to the solution and the pH determined before and after each addition. From these data a titration curve is drawn by plotting the amount of alkali or acid used, against the change in pH produced. The buffer value at any pH may be determined graphically by drawing the tangent to the curve at that pH and determining its slope. Mathematically, if the added alkali or acid is Δ B, and the increase in pH is Δ pH, then the average buffer value over the observed pH range is $\beta = \frac{\Delta B}{\Delta pH}$.

Van Slyke (3) was the first to adopt the differential ratio $\frac{dB}{d\,pH}$ as the expression of the relation between the increment in gram equivalents per liter of acid or base added to a buffer solution and the resultant increment in pH.

The buffer index of a solution is not constant and has an exact value only at a definite pH, and therefore the value calculated over an observed pH range is an average one. In practise, a close approximation is made if the buffer index is calculated for the mean pH over a small pH interval. The area under a buffer curve between two pH values is a measure of the buffer capacity of the solution in the particular range chosen.

EXPERIMENTAL METHOD

The initial pH of the milk was determined, and then to 50 cc. portions were added 3, 5, 7, 10, and 12 cc., respectively, of 0.2 N hydrochloric acid. The acid was added from a burette to each portion in a 250 cc. Erlenmeyer flask while it was shaken vigorously. The final pH was measured after the lapse of two hours, since Whittier (1) has shown that equilibrium is attained in this time.

The hydrogen-ion concentrations were determined by means of the quinhydrone method, using gold electrodes of the capillary form (6). The potential differences against a saturated calomel

half-cell were measured with a Leeds and Northrup type K potentiometer.

Titration curves were then plotted and the cubic centimeters of acid required for each change of 0.2 in pH were read therefrom. The buffer index for each 0.2 pH interval was then calculated by substitution in the formula,

$$\frac{d\,B}{d\,pH} = \frac{\text{(cc. acid added) (normality factor)}}{\text{(volume of milk) (pH change)}}$$

Van Slyke (3) states that the volume change due to the added acid or alkali may be ordinarily neglected, if the maximum increase is below 50 per cent of the original volume. Then too, the work of Buchanan and Petereson (5) indicates that milk may be diluted up to 30 per cent of its volume without appreciable effect on the buffer value. Inasmuch as the volume changes which occurred in the present work were below these limits, no correction for them was considered necessary in the above formula.

A number of determinations were made over the pH range between 4.5 and 6.5 in order to locate the region of maximum buffering; typical curves are shown in figure 1. It was found that the maximum buffer values occurred between pH 5 and 6, and consequently most of the determinations were limited to this zone.

DESCRIPTION OF MILK

The milk on which these buffer studies were made came from the herd at the Bureau of Dairy Industry Experiment Station at Beltsville, Maryland. The herd consisted of approximately 25 Jersey and 25 Holstein cows, but the milk received was not always from the same cows each day. The samples taken for the determinations on the Jersey and Holstein milk represented about 550 pounds of milk, and the samples taken of the mixtures of these milks represented about 1100 pounds.

The samples were obtained after the milk had been clarified, standardized, and thoroughly mixed for the purpose of Swiss cheese manufacture. The standardization was done by separating

some of the fat from a portion so that the ratio of fat to casein was about 1:7. Inasmuch as fat is a negligible factor in buffering, change in the percentage of it would not have an appreciable effect upon the buffer values.

The initial pH of the milk samples ranged from 6.50 to 6.64.

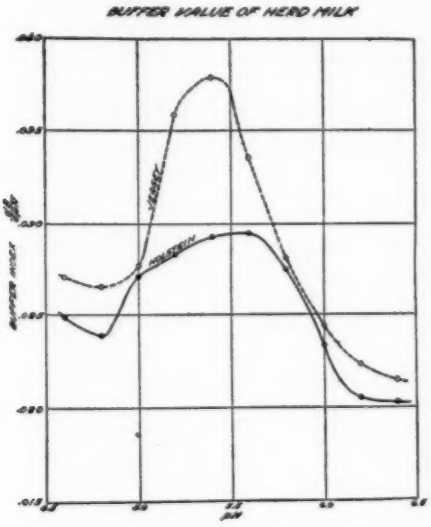

FIG. 1

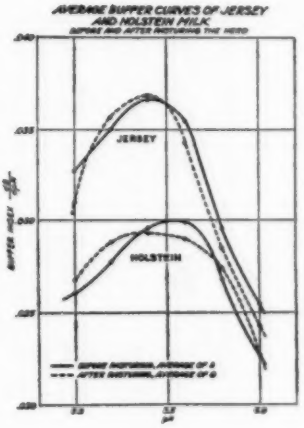

FIG. 2

DIFFERENCE IN THE BUFFER VALUE OF JERSEY AND HOLSTEIN MILK AND THE EFFECT OF PASTURING THE HERD

The experiments of some investigators (7) (8), indicate that the change in rations occurring when cows are pastured, results in an increase in those milk constituents of importance in buffering, such as protein and ash, while from a review of other researches (9), it may be concluded that changes in ration are without significant effect upon these milk constituents. Some determinations were made of the buffer value of Holstein and Jersey milk before and after the herd was pastured. These results are presented in tables 1 and 2, and the average values are plotted

TABLE 1

Buffer values of Jersey and Holstein milk before the herd was pastured

pH zone	February 11, 1929		February 12, 1929		February 13, 1929		February 14, 1929		February 15, 1929		February 16, 1929		February 18, 1929		April 22, 1929		April 23, 1929	
	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein
4.7–4.9		0.0293		0.0257		0.0222		0.0220		0.0234		0.0228		0.0224	0.0227	0.0277	0.0401	0.0251
4.9–5.1	0.0339	0.0251	0.0297	0.0277	0.0369	0.0265	0.0335	0.0241	0.0353	0.0259	0.0351	0.0265	0.0301	0.0259	0.0311	0.0261	0.0289	0.0273
5.1–5.3	0.0371	0.0257	0.0351	0.0281	0.0343	0.0289	0.0341	0.0271	0.0351	0.0275	0.0351	0.0305	0.0343	0.0269	0.0351	0.0283	0.0347	0.0275
5.3–5.5	0.0373	0.0319	0.0363	0.0285	0.0351	0.0295	0.0359	0.0313	0.0359	0.0281	0.0355	0.0293	0.0387	0.0285	0.0375	0.0293	0.0381	0.0297
5.5–5.7	0.0361	0.0313	0.0365	0.0283	0.0347	0.0293	0.0359	0.0327	0.0357	0.0283	0.0367	0.0287	0.0337	0.0303	0.0335	0.0299	0.0369	0.0301
5.7–5.9	0.0307	0.0255	0.0299	0.0255	0.0309	0.0281	0.0293	0.0249	0.0303	0.0267	0.0291	0.0293	0.0299	0.0271	0.0301	0.0277	0.0265	0.0265
5.9–6.1	0.0251	0.0227	0.0257	0.0237	0.0259	0.0233	0.0253	0.0218	0.0253	0.0228	0.0253	0.0214	0.0271	0.0220	0.0245	0.0227	0.0255	0.0243

TABLE 2

Buffer values of Jersey and Holstein milk after the herd was pastured (April 23, 1929)

pH zone	April 24, 1929		April 25, 1929		April 26, 1929		April 27, 1929		April 29, 1929		April 30, 1929		May 2, 1929		May 3, 1929	
	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein
4.7–4.9		0.0261		0.0244		0.0251	0.0265	0.0239		0.0259	0.0261	0.0241		0.0222		0.0224
4.9–5.1	0.0333	0.0281	0.0321	0.0271	0.0363	0 0271	0.0277	0.0271	0.0317	0.0277	0.0299	0.0255	0.0279	0.0261	0.0289	0.0257
5.1–5.3	0.0389	0.0309	0.0361	0.0285	0.0367	0.0299	0.0359	0.0283	0.0371	0.0305	0.0329	0.0291	0.0359	0.0277	0.0317	0.0257
5.3–5.5	0.0411	0.0315	0.0375	0.0289	0.0363	0.0315	0.0379	0.0293	0.0377	0.0299	0.0343	0.0305	0.0377	0.0259	0.0329	0.0269
5.5–5.7	0.0325	0.0307	0.0361	0.0287	0.0351	0.0321	0.0335	0.0295	0.0321	0.0289	0.0367	0.0297	0.0335	0.0251	0.0347	0.0275
5.7–5.9	0.0319	0.0267	0.0277	0.0283	0.0287	0.0269	0.0281	0.0275	0.0279	0.0253	0.0307	0.0303	0.0275	0.0291	0.0255	0.0251
5.9–6.1	0.0244	0.0220	0.0238	0.0220	0.0253	0.0234	0.0245	0.0233	0.0255	0.0228	0.0243	0.0233	0.0238	0.0246	0.0228	0.0200

in figure 2. It is evident that after the herd was pastured there was a small shift of the peak of the buffer values to the left (possibly indicating a small change in the milk constituents), but the effect was slight, the marked deviation in buffer index being due to the difference in breed.

In connection with the difference in the buffer values of Holstein and Jersey milk, it is interesting to note that there also exists a difference in the hardness of the curd of milk from the two

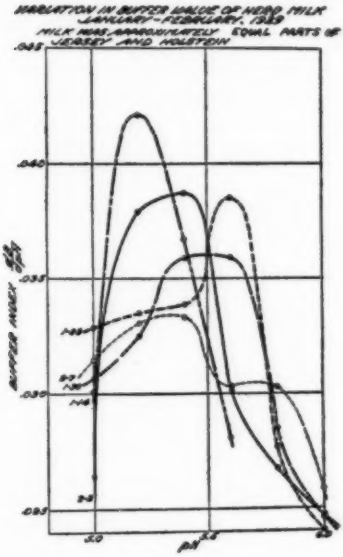

FIG. 3

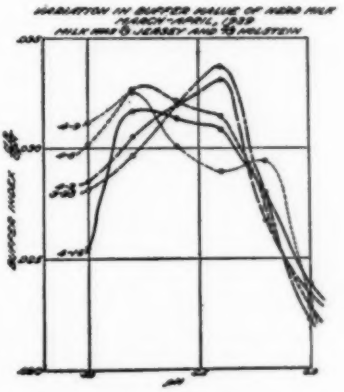

FIG. 4

breeds, which is of practical significance in nutrition and the dairy industries. Hill (10), who studied the physical curd character of milk in its relation to digestibility for infants, found that Holstein milk was much softer in curd character than Jersey milk, on the average. These results have been corroborated in these laboratories by Mr. K. J. Matheson. Then too, Holm and Webb (2) have pointed out that cow's milk in order to simulate the action of mother's milk in addition to yielding a fine soft curd,

TABLE 3

Buffer value of herd milk

Milks were a mixture of approximately one-half Jersey and one-half Holstein

pH zone	JANUARY 28, 1929	JANUARY 29, 1929	JANUARY 30, 1929	JANUARY 31, 1929	FEBRUARY 1, 1929	FEBRUARY 2, 1929	FEBRUARY 4, 1929	FEBRUARY 6, 1929	FEBRUARY 7, 1929	FEBRUARY 8, 1929	FEBRUARY 9, 1929	AVERAGE
4.9–5.1	0.0329	0.0293	0.0307	0.0319	0.0321	0.0265	0.0295	0.0313	0.0315	0.0269	0.0309	0.0303
5.1–5.3	0.0335	0.0315	0.0325	0.0377	0.0333	0.0379	0.0315	0.0357	0.0331	0.0387	0.0357	0.0346
5.3–5.5	0.0339	0.0343	0.0359	0.0357	0.0337	0.0387	0.0361	0.0351	0.0333	0.0379	0.0347	0.0354
5.5–5.7	0.0385	0.0359	0.0359	0.0321	0.0335	0.0301	0.0421	0.0333	0.0303	0.0333	0.0363	0.0347
5.7–5.9	0.0277	0.0319	0.0285	0.0279	0.0297	0.0273	0.0305	0.0313	0.0303	0.0295	0.0275	0.0293
5.9–6.1	0.0241	0.0261	0.0247	0.0255	0.0255	0.0247	0.0253	0.0253	0.0257	0.0249	0.0249	0.0252

TABLE 4

Buffer value of herd milk

Milks were a mixture of one-third Jersey and two-thirds Holstein in exact proportion

pH zone	MARCH 28, 1929	MARCH 30, 1929	APRIL 1, 1929	APRIL 2, 1929	APRIL 5, 1929	APRIL 6, 1929	APRIL 9, 1929	APRIL 12, 1929	APRIL 13, 1929	APRIL 15, 1929	APRIL 16, 1929	APRIL 19, 1929	AVERAGE
4.9–5.1	0.0281	0.0273	0.0289	0.0285	0.0277	0.0301	0.0311	0.0289	0.0273	0.0287	0.0255	0.0265	0.0282
5.1–5.3	0.0297	0.0327	0.0357	0.0305	0.0299	0.0327	0.0325	0.0309	0.0333	0.0309	0.0317	0.0321	0.0320
5.3–5.5	0.0319	0.0325	0.0327	0.0319	0.0305	0.0321	0.0301	0.0311	0.0313	0.0303	0.0313	0.0329	0.0316
5.5–5.7	0.0337	0.0309	0.0287	0.0331	0.0291	0.0315	0.0289	0.0319	0.0285	0.0311	0.0309	0.0333	0.0308
5.7–5.9	0.0277	0.0259	0.0263	0.0267	0.0267	0.0273	0.0295	0.0287	0.0287	0.0289	0.0279	0.0257	0.0275
5.9–6.1	0.0222	0.0228	0.0234	0.0234	0.0234	0.0228	0.0230	0.0232	0.0228	0.0230	0.0238	0.0243	0.0232

should have a low buffer capacity, the first point being the more important. It is apparent that if Jersey milk were diluted so the solids-not-fat content would be the same as in Holstein milk, then the buffer capacity of the two milks should not be markedly different. Furthermore, in the field of nutrition, the curdling properties of milk may be more important than the buffer value (2). Then too, the difference between the hardness of the curd of Jersey milk and Holstein milk would probably be less if the protein concentration of the milks were equalized.

The average initial pH of the Jersey milk was 6.56 as compared with 6.58 pH for the Holstein milk. The fact that the difference between the pH of the milks was small indicates that there is no correlation between the buffer value and the hydrogen-ion concentration of milk.

VARIATION IN THE BUFFER VALUE OF MIXTURES OF JERSEY AND HOLSTEIN MILK

In table 3 are some buffer values of milk received in January and February. This milk was mixed at the farm, and was approximately half Jersey and half Holstein milk. Some of the results are shown graphically in figure 3; the curves serve to indicate the variation in buffer indices which are likely to occur from day to day in a milk supply.

A number of experiments were made in which the buffer capacity was determined on milks which were composed of exactly one-third Jersey and two-thirds Holstein milk, the proportions being accurately determined in the laboratory. These results are shown in table 4, and some are plotted in figure 4. It will be noted that daily variations in the buffer value also occurred when the proportions of the two milks were determined exactly. The decreased percentage of Jersey milk accounted for the lower buffer value of these milks, the average of the peaks falling at about 0.032, whereas in the previous experiment the average value was above 0.035.

SUMMARY

Jersey milk was found to have a markedly greater buffer capacity than Holstein milk, in the pH zone of 5 to 6.

Pasturing the herd had a negligible influence upon the buffer value of the milk.

The extent of the variations in buffer value which are likely to occur in a milk supply are indicated.

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VIABILITY OF LACTOBACILLUS ACIDOPHILUS AND LACTOBACILLUS BULGARICUS CULTURES STORED AT VARIOUS TEMPERATURES*

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Investigators have found that a large number of living acidophilus organisms must be ingested in order to secure beneficial results from the consumption of acidophilus preparations. According to Kopeloff (1), about 1000 cc. of milk containing 200 million viable acidophilus organisms per cubic centimeter should be ingested daily. The therapeutic effect was found to decrease when the number of living organisms was less than 100 million per cubic centimeter and only 1000 cc. of the milk was ingested each day. It is desirable therefore, that acidophilus preparations should originally contain a large number of living bacteria, and that the product should be used before there is a decided decrease in numbers.

The majority of commercial products that have been offered to the public have been unsatisfactory. Analysis (2) (3) of such commercial products has suggested that the preparations either do not originally contain a sufficiently large number of the organisms or else too long a time has elapsed between production and consumption. Since a comparatively small amount of the commercial culture is consumed daily, the presence of large numbers of acidophilus organisms is essential.

Milk cultures of *Lactobacillus acidophilus* are desirable, in that the organisms grow readily in milk and the resulting product is usually more palatable and nutritious than other forms of acidophilus products. The necessary numbers of acidophilus organisms are present in a properly prepared fresh milk culture.

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In many places the consumption of acidophilus milk is not sufficient to warrant its preparation each day. Consequently, these cultures may be prepared once or twice a week. This necessitates storage of the acidophilus culture for varying periods of time before its distribution. In addition the cultures are kept in the home for a time before the product is entirely consumed. For these reasons the acidophilus preparations may be several days old before they are consumed.

Personal experience, as well as the experience of others, indicates that cultured milks are more palatable if they are consumed when they are cold. When a milk culture is kept at room temperatures, it is less palatable. In some cases this is due to the slight caramelization caused by sterilization of the milk before inoculation. This caramelized flavor is less noticeable in a chilled product. In many instances a slight caramelization of the milk is not at all apparent in cold acidophilus milk. A "chalky" flavor has also been noticed in warm cultured milks just as they were taken from the incubator. This flavor disappeared upon cooling of the milk below room temperature.

When cultured milks of the proper acidity are kept for a time at refrigerator temperatures, they retain their desirable, mildly acid flavor. At room temperatures, the acidity of the culture increases and so causes the milk to be more acid to the taste than is desirable. In addition, there is more "wheying off" of the milk, resulting in a product of less desirable appearance. If the culture is kept for several days, the acidity may increase sufficiently to make the milk unfit for consumption. When the acidity becomes too high a diminution of the desired organisms also results. Since a large number of organisms is necesary, this action is detrimental.

Results showing a great loss in numbers when acidophilus milk was kept in the refrigerator have frequently been quoted in the recent literature on *L. acidophilus*. For this reason, it has been recommended that acidophilus products be stored at room temperatures. If the organisms die rapidly at refrigerator temperatures, keeping the product at cold temperatures would retain palatability but would result in a loss of therapeutic proper-

ties. In keeping the product at warmer temperatures, a less palatable product would result, with the probability of lessened consumption.

Results that show a marked reduction in the number of viable organisms when acidophilus milk is kept at refrigerator temperatures, thus assume considerable importance. Such results are also contrary to the accepted effects of low temperatures upon bacterial life.

HISTORICAL

Kopeloff and Cheney (4) inoculated 3 flasks of sterilized milk with *L. acidophilus* and after incubation at 37°C. for 24 hours, the flasks were held at 25° to 30°C. for two weeks. They reported that the number of viable organisms diminished rapidly after 3 days, accompanied by a corresponding rise in acidity. The milk became less palatable and had a very unpleasant taste by the end of two weeks. Similar results were obtained upon repetition of the experiment.

In further experiments Kopeloff (1) inoculated 3 flasks, each containing 300 cc. of sterilized skim milk, with 10 cc. of culture per flask. The flasks were incubated at 35°C. for 24 hours, and then placed in an ice box. The number of viable *L. acidophilus* was determined each day by plating a portion of the milk. After one day in the ice box, 46 per cent of the viable organisms had died; after 2 days, 73; and after 3 days, 89 per cent of the original number were not viable. Kopeloff concluded that it is more desirable to keep *L. acidophilus* products at room temperature than at ice box temperatures.

Reichart and Davis (5) also presented some counts on acidophilus milk stored at various temperatures. When held at 2° to 15°C. the number of organisms in acidophilus milk was found to decrease considerably. In later experiments there was little decrease in number of organisms during the first 24 hours storage at 24°C. and in two cases there was an increase. During the second 24 hours there was approximately a 50 per cent decrease at 24°C. At 4°C. there was a decided decrease, as was the case at 37°C. It was concluded that 21°C. was better for storing

acidophilus milk than refrigerator temperatures. The storage of acidophilus milk for longer than 48 hours, even at room temperature, was found to result in a product containing few *L. acidophilus* organisms.

PROCEDURE

The cultures of *L. acidophilus* and *L. bulgaricus* used in the following experiments were secured from our stock cultures or from the collections of other investigators. Culture A1, A4, and B6 were stock cultures. Culture A15 was a recently isolated fecal strain. These cultures were transferred into tubes of sterile milk at 24-hour intervals for a few days preceding their use.

Separated milk of a low bacterial content was sterilized at 15 pounds pressure for 25 minutes. After cooling, a flask was inoculated with 1 per cent of a 24-hour milk culture of *L. acidophilus*. The flask was then placed at 37°C. for incubation until there was evidence of coagulation. The increase in acidity was determined at intervals by withdrawing a sample for titration. The flasks were removed from the incubator after coagulation took place or when the acidity reached a predetermined amount.

A sample was withdrawn for plating from the well mixed culture. A portion of the culture was then placed into each of 4 sterilized flasks, stoppered with cotton. One of these flasks was placed at each of the different temperatures used, 37°, 22°, 9° and 0°C. These cultures were held at the respective temperatures for one week. Samples for plating and for acidity were withdrawn every 24 hours.

The medium used for all plating was Bacto dehydrated whey agar. Duplicate plates were poured and incubated at 37°C. for 5 days. The dilutions were made in rubber-stoppered, glass bottles containing an amount of distilled water determined by experiment to be 99 cc. after sterilization. The use of a solid stopper permitted thorough shaking of the samples, a procedure essential for breaking up the coagulated milk. In the majority of the experiments, only one dilution (1:1,000,000) was made.

A 10 cc. sample was used for the acidity titration. Straight walled glass tubing, marked to contain 10 cc., or inverted 10 cc.

pipettes, similarly marked, were satisfactory for measuring the coagulated milk for the acidity test.

Samples for the acidity test were withdrawn from the well stirred sample by submerging only slightly the bottom of the sampling pipette, and drawing the milk just up to the mark. In this way, very little of the culture remained on the outside of the pipette or inside the pipette above the mark. It was necessary to rinse out all of the milk within the pipette. The simplest way to do this seemed to be to use the pipette as a stirring rod during the titration of its respective sample. After diluting the milk by the addition of alkali, the pipette could be well rinsed with the alkali and milk mixture. A more rapid titration was also possible, inasmuch as alkali could be rapidly added to slightly past the endpoint. Then, by rinsing out the remaining culture in the pipette, the reaction would again be acid and the endpoint could then be carefully approached.

PRESENTATION OF DATA

In a preliminary experiment on *L. acidophilus*, culture A15, a flask of the culture was placed in storage at 0°C. After two days the count had decreased from 1075 million to 820 million organisms per cubic centimeter.

This result was interesting, inasmuch as two days storage at 0°C. had reduced the living organisms less than 25 per cent.

The results of 8 experiments at intervals over a period of 7 days are given in tables 1 and 2. The cultures in these tables are grouped by culture number, and not in order of the experiment.

Minor fluctuations in the percentage of acidity sometimes appeared. These were slight and probably were largely due to the approximate measurements secured with the sampling tubes marked to contain 10 cc.

Considerable fluctuations were secured in some of the *L. acidophilus* counts. While the fact that the bacteria may be clumped may account for some fluctuation, yet other factors are concerned. The fluctuations seemed to be due to failure of the organisms to grow as colonies on the media and under the conditions provided. That they were present in the culture and viable,

TABLE 1

The acidity and count of L. acidophilus milk cultures stored at the temperatures indicated

CULTURE	STORAGE	0°C.		9°C.		22°C.		37°C.	
		Acidity	Count	Acidity	Count	Acidity	Count	Acidity	Count
	days	*per cent*	*millions*	*per cent*	*millions*	*per cent*	*millions*	*per cent*	*millions*
A1	0	0.50	88.0*	0.50	88.0*	0.56	88.0*	0.56	88.0*
	1	0.58	21.0	0.59	31.0	0.72	84.0	1.08	46.5
	2	0.58	30.5	0.61	109.0	0.73	†	1.40	0.5
	3	0.59	131.0	0.60	106.0	0.72	100.0	1.40	0.0
	4	0.58	28.0	0.61	97.0*	0.75	146.5	1.41	0.0
	5	0.59	74.5	0.58	73.5	0.86	113.0	1.47	0.0
	6	0.66	107.0	0.63	99.0*	0.86	124.0*	1.48	0.0
	7	0.59	8.0*	0.63	54.0	0.93	38.5	1.53	0.0
A1	0	0.80	118.0	0.80	118.0	0.80	118.0	0.80	118.0
	1	0.81	166.5	0.83	137.0	1.04	125.5	1.41	75.0
	2	0.81	93.5	0.85	119.5	1.18	127.5	1.69	67.0
	3	0.81	73.5	0.83	†	1.22	108.5	1.81	44.0
	4	0.81	59.5	0.85	118.0	1.24	83.0	1.86	38.0
	5	0.81	53.5	0.85	135.5	1.27	108.0*	1.96	13.5
	6	0.81	68.5	0.85	106.0*	1.29	94.0	1.96	8.0
	7	0.81	56.5	0.87	64.5	1.37	66.0*	2.07	5.0
A1	0	0.78	50.5	0.78	50.5	0.78	50.5	0.78	50.5
	1	0.75	65.0	0.78	53.0*	1.01	59.5	1.40	55.5
	2	0.78	42.0	0.84	41.5	1.06	42.5	1.62	34.0
	3	0.78	39.0*	0.85	38.0	1.16	†	1.79	15.0
	4	0.77	36.0*	0.85	37.5	1.21	51.5	1.90	4.0
	5	0.78	56.5	0.85	43.5	1.24	38.5	2.02	1.5
	6	0.78	39.0*	0.86	47.5	1.29	43.0	2.08	1.0
	7	0.78	44.0	0.86	31.0	1.35	16.5	2.16	0.0
A4	0	0.79	135.5	0.79	135.5	0.79	135.5	0.79	135.5
	1	0.81	16.5	0.81	130.0	1.00	285.0	1.31	188.0
	2	0.81	138.0	0.83	255.0*	1.01	45.0	1.55	0.0
	3	0.81	329.0	0.82	324.0	1.03	239.0*	1.61	0.0
	4	0.80	257.0	0.82	291.0*	1.04	200.5	1.66	0.5
	5	0.78	20.0*	0.77	148.5	1.10	185.0*	1.80	0.0
	6	0.80	107.0	0.86	46.5	1.19	217.5	1.71	0.0
	7	0.79	44.5	0.86	113.0	1.19	3.5	1.72	0.0
A15	0	0.80	1,035.0	0.80	1,035.0	0.80	1,035.0	0.80	1,035.0
	1	0.80	705.0	0.85	1,105.0	0.95	1,835.0	1.37	2,140.0
	2	0.83	985.0	0.85	1,205.0	1.03	2,160.0	1.59	1,605.0
	3	0.80	960.0	0.81	1,335.0	1.06	1,775.0	1.96	1,630.0
	4	0.79	745.0	0.94	1,300.0	1.31	1,900.0	2.11	1,820.0
	5	0.78	985.0	0.94	1,380.0*	1.40	2,115.0	2.37	1,055.0
	6	0.78	990.0*	0.95	1,020.0	1.42	1,415.0	2.31	505.0
	7	0.79	680.0	1.01	1,195.0	1.51	2,000.0*	2.32	56.5

* Result of one plate.

† No count, usually spreaders.

is demonstrated by their appearance in increased numbers on days following those in which the count was low. The fact that

TABLE 2

The acidity and count of L. bulgaricus milk cultures stored at the temperatures indicated

CULTURE	STORAGE	0°C.		9°C.		22°C.		37°C.	
		Acidity	Count	Acidity	Count	Acidity	Count	Acidity	Count
	days	*per cent*	*millions*	*per cent*	*millions*	*per cent*	*millions*	*per cent*	*millions*
B6	0	0.83	221.5	0.83	221.5	0.83	221.5	0.83	221.5
	1	0.85	†	0.85	251.0	0.92	162.0	1.48	265.0
	2	0.84	345.0	0.86	324.5	0.90	198.0	1.78	0.5
	3	0.85	265.5	0.86	314.5	0.92	208.5	1.85	1.5
	4	0.84	236.0*	0.86	322.5	0.92	262.0	1.91	0.0
	5	0.83	291.0	0.83	399.0	0.90	317.5	1.93	0.0
	6	0.83	226.0*	0.83	311.5	0.95	398.0*	2.00	0.0
	7	0.83	188.5	0.86	187.0	0.93	176.0	2.00	0.0
B6	0	0.71	155.5	0.71	115.5	0.71	155.5	0.71	115.5
	1	0.71	46.0	0.73	111.0	0.86	108.0	0.98	10.0
	2	0.70	41.5	0.74	74.0	1.01	81.0	1.04	1.0
	3	0.70	22.5	0.73	58.5	0.99	46.5	1.11	1.5
	4	0.71	18.0*	0.75	71.5	0.99	37.5	1.08	3.0
	5	0.71	9.5	0.76	56.5	0.99	33.0	1.15	3.0*
	6	0.70	11.5	0.78	39.5	1.00	9.0*	1.15	2.5
	7	0.71	6.0	0.79	13.0	1.01	4.5	1.20	2.0
B6	0	0.63	201.5	0.63	201.5	0.63	201.5	0.63	201.5
	1	0.59	162.5	0.68	200.0	0.78	221.5	1.16	136.0
	2	0.63	167.5	0.77	207.5	0.90	180.5	1.40	77.0*
	3	0.65	†	0.74	200.5	0.93	229.0*	1.52	42.0*
	4	0.65	198.0	0.73	222.0	0.99	220.0	1.58	92.5
	5	0.65	206.0	0.76	229.5	1.08	233.5	1.65	96.0
	6	0.65	193.5	0.78	216.5	1.15	208.5	1.75	71.5
	7	0.65	123.0	0.82	109.0	1.16	149.0	1.86	27.5

* Result of one plate.

† No count, spreaders or molds.

higher counts are secured after low counts is a positive indication of the presence of the organisms.

Apart from the fluctuations in plate counts of the acidophilus organisms, very definite trends are indicated with respect to the

change in numbers of viable organisms at the different temperatures. This may perhaps be more apparent from the summary presented in table 3. This table indicates the viable organisms present, expressed in percentage of the original number before storage.

According to the results of the experiments, as shown in tables 1, 2, and 3, there is only a slight reduction in the numbers of viable organisms upon holding cultured milks at refrigerator temperatures, even during a period of one week. As a matter of interest, storage of these products at as low a temperature as 0°C. did not

TABLE 3

Summary of the effect of storage of L. acidophilus milk upon the number of organisms

Results expressed as percentage of original count before storage

DAYS OF STORAGE	0°C.				9°C.				22°C.				37°C.			
	Average			Average all strains	Average			Average all strains	Average			Average all strains	Average			Average all strains
	A1	A4	A15		A1	A4	A15		A1	A4	A15		A1	A4	A15	
1	97	12	68	74	85	95	106	91	106	210	117	141	75	138	206	114
2	65	101	95	78	102	118	116	108	96	33	208	106	41	0	155	55
3	96	242	92	124	97	239	128	132	102	176	171	131	22	0	157	44
4	51	189	71	83	94	214	125	124	112	147	183	134	13	0	175	43
5	80	14	95	80	94	109	133	105	98	136	204	127	4	0	101	23
6	85	78	95	86	98	34	98	85	101	160	136	120	2	0	48	11
7	48	32	65	48	59	83	115	75	44	2	193	65	1	0	5	1

result in the diminution of organisms to the extent reported by other investigators as a result of storage at refrigerator temperatures.

There was very little difference in the percentage decrease, whether stored at room or refrigerator temperatures. However, the product held in the refrigerator retained its palatability and keeping quality, increasing only slightly in acidity. The product kept at room temperature increased considerably in acidity, with a consequent loss in palatability and keeping quality. Furthermore, the room temperature used was constantly 22°C., a lower temperature than would be maintained in the average room, par-

ticularly in summer. At temperatures of 37°C., a high acidity was soon reached and the number of viable organisms was quickly reduced, resulting in a loss of therapeutic properties, keeping quality and palatability.

Comparison of L. acidophilus and L. bulgaricus

In table 4 the average of three experiments on *L. acidophilus* is compared to the average of three experiments on *L. bulgaricus*.

The results given in table 4 indicate that *L. acidophilus* and *L. bulgaricus* are similar in their ability to remain viable in milk cultures when held at the temperatures indicated.

TABLE 4

A comparison of the effect of storage upon L. acidophilus and upon L. bulgaricus

Results expressed as percentages of original count before storage

DAYS OF STORAGE	AVERAGE 3 TRIALS L. ACIDOPHILUS, STRAIN 1				AVERAGE 3 TRIALS L. BULGARICUS, STRAIN 6			
	0°C.	9°C.	22°C.	37°C.	0°C.	9°C.	22°C.	37°C.
1	97	85	106	75	55	94	84	64
2	65	102	96	41	88	99	67	13
3	96	97	102	22	44	93	79	7
4	51	94	112	13	72	100	83	15
5	80	94	98	4	79	110	93	16
6	85	98	101	2	68	91	96	12
7	48	59	44	1	49	62	52	5

The storage of an L. acidophilus commercial broth culture

In the results already presented, only milk cultures have been concerned. One analysis made on a commercial broth preparation of *L. acidophilus* is also of interest in connection with the temperature of storage. According to the label, the organism used was predominately an X strain, recently isolated from the human intestinal tract. This culture was regularly purchased from a laboratory specializing in these cultures.

According to the label, the preparation examined was manufactured November 1, 1929. The number of viable organisms at the time of manufacture was stated to be 200,000,000 per cubic

centimeter. The expiration date was December 1, 1929. The estimated number of viable organisms at the date of expiration was stated to be 100,000,000 per cubic centimeter. The label also stated that the preparation should be kept at room temperature.

This product was plated out on November 4 and again on November 14. At this latter time the preparation was divided, a portion being placed in the refrigerator 9°C. (48°F.) and the remainder left at room temperature, 20° to 24°C. (68 to 75°F.). The results obtained are presented in table 5.

The results obtained with the broth culture indicate again that refrigerator temperature is equal or superior to room temperature for the storage of *L. acidophilus*.

TABLE 5

Counts obtained upon a commercial broth culture of L. acidophilus

DATE EXAMINED	TEMPERATURE OF STORAGE	PLATE COUNT
November 4, 1929	Room	48,000,000
November 14, 1929	Room	31,850,000
December 1, 1929	Room	3,605,000
	Refrigerator since November 14, 1929	15,150,000

Storage temperature for laboratory cultures

Still further evidence might be brought forward regarding the effect of refrigerator temperature upon *L. acidophilus*. Our stock cultures are kept at this temperature and transferred not more than once a month, unless they are needed for some special purpose.

In addition to stock cultures which might have adapted themselves to the conditions under which they are kept, similar results were obtained with a recently isolated fecal strain. Several sealed tubes of this X type recently isolated fecal strain, single cell cultures, were received in November 1928. One tube was opened at that time and used in early experiments. The other tubes were placed in the refrigerator. In November, 1929, a second sealed tube of this set which had been in the refrigerator approximately a year, was opened. The contents, 2 or 3 cc., placed in 10 cc. of sterile milk coagulated the milk within 24 hours.

DISCUSSION

The rapid decrease in the number of living organisms in milk cultures of *L. acidophilus* that has been found by other investigators is believed to be largely due to the acid formed. In the report of Kopeloff and Cheney (4) with cultures held at room temperature, the acidity was given. The acidity reported for the first, second, and third days was 1.00, 0.85, and 0.95 per cents respectively, with counts of 177, 184, and 141 millions. These investigators stated that the acidity rapidly increased thereafter, accompanied by a rapid decrease in the number of living organisms. In the work reported by Kopeloff (1) the acidity of the samples when placed in the refrigerator was not given. However, a 3⅓ per cent inoculation with a 24 hour incubation period was used. In the present investigation a 1 per cent inoculation produced about 0.8 per cent acid in 30 hours. The acid produced within a given time will depend upon the strain, its activity, amount of inoculation, time and temperature of incubation, and temperature of the milk when inoculated.

Reichart and Davis (5) did not report the acidity of the samples that they used. In the directions these investigators gave for the preparation of acidophilus milk, an inoculation with 1 to 4 per cent was used, followed by incubation for 18 to 20 hours or until a slight amount of whey was formed. The acidity at that time was stated to be from 1 to 1.5 per cent. These investigators found that acidophilus milk held at 40°F. showed a decided decrease in living organisms, during the first 24 hours, as did that held at 98°F. Acidophilus milk held at 75°F. showed only a slight decrease in 24 hours. In two cases there was an increase in numbers. Storage longer than 48 hours, even at room temperature, was found to cause a decided decrease in numbers.

According to the results of the present investigation and other unreported results, the earlier observations just mentioned can at least be partially explained upon the basis of acidity. After a certain acidity is reached, the organisms seem to die rapidly. If a sample of cultured milk reached the acidity at which a noticeable decrease in numbers began and was then divided and placed at various temperatures, the following might be expected.

At 37°C. there would be a rapid increase in acidity and a high death rate, offsetting any earlier increase in numbers. At 22°C., a slower increase in acidity would be expected, with continued death of organisms, but with a multiplication of organisms sufficient to slightly increase or nearly maintain the original number or to decidedly decrease the reduction in numbers. At 9°C. some decrease in numbers, due to the acidity already reached, would continue for a while. This decrease might largely take place during the time required to thoroughly chill the culture, and the decrease would probably be greater due to additional acid produced before the formation of acid entirely ceased. While some multiplication of organisms might occur during the cooling process, there would not be the multiplication present that occurs at 22°C. or above. The decrease in numbers due to acidity, associated with the lack of multiplication at 9°C., might result in apparently a more rapid decrease at 9°C. than at 22°C.

Observations indicate that the acidity preferred by the consumer of cultured milks seems to be not more than 0.80 to 1.00 per cent. When the milk is consumed, which may be several days after its manufacture, there will have been some increase in acidity. This increase in acid depends upon the time the product was exposed to higher temperatures during delivery, the temperatures at which it was stored where manufactured and the time and temperature at which it was held in the home before consumption. In order to have a product not too high in acidity when consumed, incubation of the milk should cease at the lowest acidity that results in a desirable acid flavor and at the same time yields a sufficient number of living acidophilus organisms.

The demand for acidophilus milk sometimes is not sufficient to warrant its preparation each day. Even if the product is made each day, some time elapses before it is consumed. In the home, the milk may not be used immediately but may be consumed over a period of a day or two. In order to secure keeping quality for such a length of time, the product must be held at refrigerator temperatures. A recommendation of room temperature for storage would result in keeping the product at a variable temperature between 22° and 37°C. depending upon seasonal or other con-

ditions. Such a temperature would impair the keeping qualities and palatability, and in addition would cause a more rapid reduction in organisms than would occur at refrigerator temperatures.

The results reported demonstrate that storage at refrigerator temperatures is the most satisfactory from the standpoint of numbers, as well as acidity. Other unpublished results and observations have also indicated that this is true.

Some of the counts on acidophilus milk reported in the various tables are lower than is desirable for therapeutic purposes. All plates in the experiments reported upon were incubated under atmospheric conditions. This might result in a lower plate count than the number of viable organisms actually present, since investigators (6) (5) (3) have shown that incubation in a carbon dioxide atmosphere results in higher plate counts. It was also intended that the cultures should be placed in storage at an acidity low enough so the acid formed would not have any destructive effect on the chilled cultures. It is probable that the cultures were removed from the incubator before the maximum number of organisms had been reached.

The different strains seem to vary somewhat in numbers. One strain gave an unusually high count. This may have been due to the fact that it was more recently isolated. On the other hand, this characteristic has been maintained for over a year as a stock culture. The same strain may vary from time to time in rapidity of coagulation and in numbers of organisms. Repeated transfers usually increase the rapidity of coagulation and increase the numbers of organisms present.

SUMMARY

1. Milk cultures of *L. acidophilus* have been stored at refrigerator temperatures for several days without causing any marked reduction in the number of living organisms.

2. Less reduction in numbers of *L. acidophilus* occurred at refrigerator temperature, 9°C. (48°F.), or even at 0°C. (32°F.), than occurred at 37°C. (99°F.).

3. There was little difference in viability between organisms in

acidophilus milk kept several days at refrigerator temperature, 9°C. (48°F.), and at 22°C. (72°F).

4. Judging from the results obtained at 9° and 0°C. it seems probable that cultured milk could be held at the usual storage temperatures of 4.4°C. (40°F.) or below for several days without greatly affecting the number of viable acidophilus organisms.

5. The various strains of *L. acidophilus* examined reacted in a similar manner to the different storage temperatures.

6. The strain of *L. bulgaricus* examined reacted to the storage temperatures in a manner similar to *L. acidophilus*.

7. The stock cultures of *L. acidophilus* and the recently isolated fecal strain used in the experiments were alike in their reactions to the storage temperatures.

8. Results of one acidophilus commercial broth culture indicated that refrigerator storage was also preferable to storage at room temperatures for that product.

9. Stock cultures of *L. acidophilus* whether old or recently isolated strains, remained viable when held in the refrigerator and transferred once in 4 to 6 weeks or longer. In one case a sealed tube of a recently isolated fecal strain stored at refrigerator temperatures for one year was viable when removed.

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CORRELATION BETWEEN ANNUAL BUTTER FAT PRODUCTION AND ANNUAL FEED COST OF DAIRY COWS UNDER FARM CONDITIONS*

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The fact that cows having a large annual milk and butterfat production are more profitable than low producers is widely recognized by dairymen and dairy workers.

Eckles and Reed (1) found that two Jersey cows raised under identical conditions of feed and care varied greatly in their ability to economically produce milk and butterfat. The better cow produced 3.9 pounds of butterfat for each pound produced by the other cow, during their first two lactations. During their third lactation the better cow produced 2.77 pounds of fat for each pound produced by the inferior cow. During this year the better cow consumed 1.75 pounds of feed for each pound consumed by the poorer cow. They found that when dry, the better cow required more feed for maintenance. The percentage of feed digested by the better cow was slightly higher than that digested by the poorer cow, but the difference was not enough to explain the difference in production.

Cooper, Bennett and Church (2) found that as the annual production of dairy cows increased there was a relatively uniform increase in the annual feed cost. As the annual production per cow increased, there was a corresponding decrease in the feed cost of a unit of production. They found this decrease greater between poor and fair cows than between fair and good cows, also a greater decrease between fair and good cows than between good and excellent cows.

Bronson (3) observed that the annual feed cost of dairy cows increased as the annual milk and fat production increased.

North (4) reported that the annual feed cost per cow was greater on farms having a higher annual production per cow.

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He also found that the feed cost per unit of production was lower on farms having a higher annual production of milk and fat per cow.

According to Erf (5) the feed cost per unit of product decreased when the annual production per cow increased. Data from the Cornell Station (6) shows that the cost of feed per unit of product is greater when the annual production is low for each cow.

Ross, Hall and Rhode (7) at Illinois found a correlation of -0.4570 ± 0.0133 between the annual butterfat production of a cow and the total digestible nutrients consumed for each pound of butterfat produced. The correlation was -0.4180 ± 0.0139 between the annual milk production per cow and the digestible nutrients consumed for each 100 pounds of milk. They found that the amount of concentrates used per pound of fat was constant but the amount of succulence and dry feed consumed for each unit of product was higher as the annual production decreased. The quantity of digestible nutrients required for each pound of fat decreased as the annual production per cow increased.

Henry and Morrison (8) point out that the production of a cow is dependent upon the feed she receives. A good cow on full feed uses only one half her ration for body maintenance and the remaining half for milk production. A cow receiving a half ration, has food only for body maintenance, and will produce little or no milk. A cow on three-quarters ration has enough feed to produce about one-half her normal flow of milk. A cow that is "over fed" produces her normal flow of milk and uses the extra feed for gain in weight.

Converse (9) was able to increase the milk and fat production of a dairy cow by feeding an excess of digestible nutrients. However the increase in production was not in exact proportion to the increase in digestible nutrients.

Dairy Herd Improvement records published in Missouri and other states all show that the higher producing cows are the more profitable ones, but little is said concerning the higher cost of feeding them.

The object of this work is to determine the correlation, if any, between the annual butterfat production of a cow and the cost

of feed for one year, regardless of the kind of feed and care the animal receives. Butterfat, instead of Milk Production is used because as Marshall (10) states "The yield of butterfat tends to constancy, the yield of milk having a negative correlation to the fat test."

METHOD OF PROCEDURE

All data used were taken from the records of the Jackson County, Missouri, Dairy Herd Improvement Association, filed

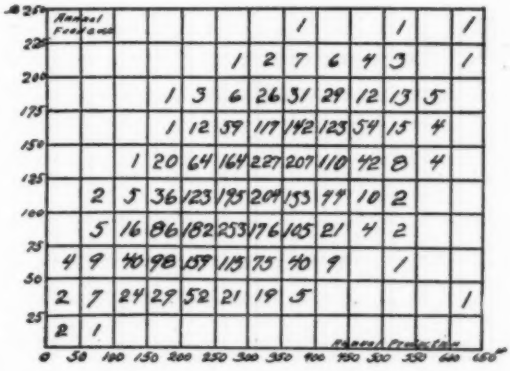

CHART 1. ORIGINAL DATA CLASSIFIED ACCORDING TO PRODUCTION AND FEED COST

Average production of 3844 cows was 307.7 ±0.96, pounds standard deviation, 87.95 ±0.67 and the coefficient of variation, 28.58 ±0.237. The average feed cost was $112.16 ±0.42, the standard deviation 38.25 ±0.30 and the coefficient of variation 34.10 ±0.291.

in the County Agrents office. These records from 79 herds, include 3844 individual yearly cow records covering a period of six years, 1923 to 1929. They were from herds of as few as 8 cows to those of over 200 and include cows of all ages. The average production of the herds ranged from 160 to 395 pounds of butterfat per cow for one year. Some were purebreds, while others were grades. Holsteins and Jerseys predominated with Guernseys and Shorthorns present in smaller numbers. Some herds were well fed and cared for while others were poorly managed. Some were farm herds, others were commercial dairy herds,

fed purchased hay and grain with no home grown feed or pasture. The herds were located in every section of the county and are a representative sample of the herds in the county.

The records of all cows, in a herd 10 months or more of the testing year, were included in these data, regardless of the length of time the animal was dry.

They were then divided into groups as shown in chart 1. The records were classified for production and feed cost, 50 pounds of butterfat being the class interval for production and $25.00 the class

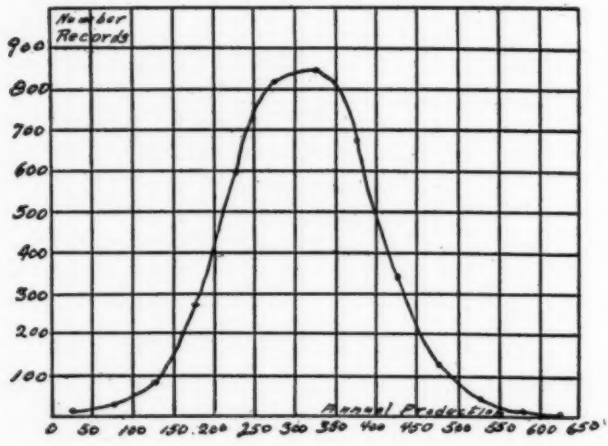

FIG. 1. FREQUENCY DISTRIBUTION OF INDIVIDUAL RECORDS ACCORDING TO THE ANNUAL BUTTERFAT PRODUCTION OF THE INDIVIDUAL COWS

interval for the annual feed cost. Frequency distribution and regression graphs were made. Correlation and other figures were all calculated by the methods given by Yule (11).

PRESENTATION OF DATA

The original data are all presented in chart 1 showing the number of records in each of the class intervals both by production and feed cost. The frequency distribution by production is practically normal (fig. 1). The frequency distribution by feed

cost (fig. 2) is more nearly a combination of two normal curves, rather than one curve. This probably is due to combining the records, from commercial dairies that buy all their feed and have a high average feed cost with records from farm herds that buy no hay and little grain and have a low average feed cost.

The average feed cost for each of the groups according to production (table 1) was found to range from $43.75 a year for the group that produced less than 50 pounds of fat annually, to

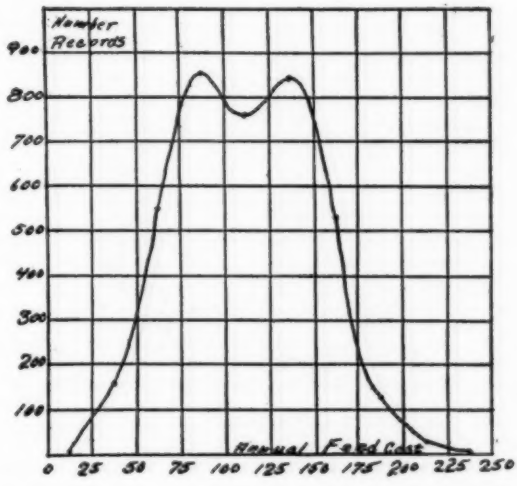

FIG. 2. FREQUENCY DISTRIBUTION OF INDIVIDUAL RECORDS ACCORDING TO THE ANNUAL FEED COST OF THE INDIVIDUAL COWS

$164.43 for the group that produced above 550 pounds and less than 600 pounds of fat. The feed cost was lower for the group that produced over 600 pounds of fat, probably due to the small number of records in this class. The average production according to feed cost (table 2) ranged from 41.6 pounds of fat annually in the group that cost less than $25.00 to feed one year, to 508.3 pounds of fat annually in the group that cost over $225.00 to feed a year. The range or variation in each of these groups was very uniform as shown by the regression graph (fig. 3) showing the average

feed cost by production and the average production by feed cost. None of the averages vary greatly from the normal line of regression.

TABLE 1

Average feed cost by production

PRODUCTION	NUMBER	ANNUAL FEED COST	FEED COST PER 1 POUND FAT
pounds			
0–50	8	$43.75	$1.75
50–100	24	63.25	0.833
100–150	86	63.07	0.505
150–200	271	80.86	0.462
200–250	595	89.01	0.396
250–300	814	105.07	0.382
300–350	846	118.24	0.364
350–400	671	128.04	0.341
400–450	342	143.79	0.338
450–500	126	152.58	0.321
500–550	45	162.50	0.310
550–600	13	164.43	0.286
600–650	3	162.50	0.259
Average.....307.7		$112.16	36.4¢
Total.....	3,844		

TABLE 2

Average production by feed cost

FEED COST	NUMBER	AVERAGE PRODUCTION
$0–25	3	41.6
25–50	160	217.5
50–75	550	232.8
75–100	850	278.1
100–125	754	303.8
125–150	847	339.7
150–175	527	376.7
175–200	126	403.2
200–225	24	425.0
225–250	3	508.3
Average....$112.16		307.7
Total.....	3,844	

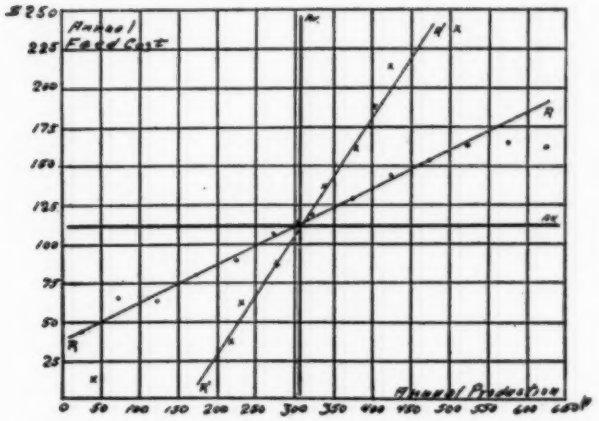

FIG. 3. CORRELATION BETWEEN YEARLY BUTTERFAT PRODUCTION AND ANNUAL FEED COST OF DAIRY COWS

Means of the rows are shown by crosses and means of the columns by dots. RR and R′R′ are lines of regression. The correlation $\gamma = +0.5641 \pm 0.007$.

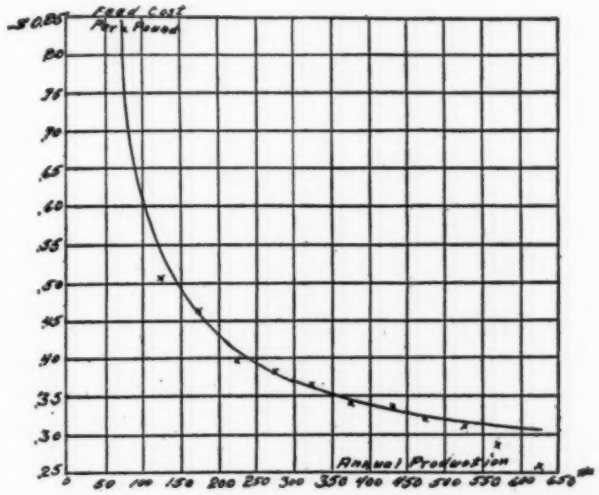

FIG. 4. VARIATION IN THE AVERAGE FEED COST OF 1 POUND OF FAT FROM COWS OF DIFFERENT PRODUCTIVITY

The data show that the feed cost of 1 pound of fat decreased as the annual production per cow increased (fig. 4 and table 1). They show definitely that the feed cost decreases rapidly as the production increases up to 400 pounds of fat annually. When yearly production is above 400 pounds of fat the decrease, in the cost of feed for each pound, is much smaller in proportion to the increase in production.

The increase in annual feed cost is smaller in proportion to the increase in production when the annual production is low. This is shown in table 3 which is a table calculated by using the re-

TABLE 3

Increase in feed cost when production is doubled

PRODUCTION	FEED COST*	DOUBLE PRODUCTION	FEED COST*	INCREASED COST	PER CENT INCREASE
pounds		*pounds*			
100	$61.21	200	$85.74	$24.53	40.1
150	73.47	300	110.27	36.80	50.1
200	85.74	400	134.80	49.06	57.2
250	98.01	500	159.33	61.32	62.6
300	110.27	600	183.86	73.59	66.7

* These feed cost figures are calculated values obtained by the use of the regression formulae given in this paper but check very closely with the actual values given in table 1.

gression equation. The feed cost was 40.1 per cent greater, for cows that produced 200 pounds of fat annually than it was for those that produced only 100 pounds. The feed for cows that produced 600 pounds of fat cost 66.7 per cent more than the feed for those that produced only 300 pounds of fat in a year.

The average yearly fat production of the 3844 cows was 307.7 $\pm$0.96 pounds with a standard deviation of 87.95 $\pm$0.67 and a coefficient of variation of 28.58 $\pm$0.237 per cent. The average annual feed cost was $112.16 $\pm$0.42 with a standard deviation of 38.25 $\pm$0.30 and a coefficient of variation of 34.10 $\pm$0.291 per cent.

The correlation between the annual fat production and the yearly feed cost was 0.5641 ±0.007. The regression equations are:

$$x = 0.2453\ y + 36.68$$
$$y = 1.296\ x + 162.3$$

When x is the annual cost of feed in dollars and y is the annual production in pounds of fat. Figure 3 shows the correlation between the annual fat production of a cow and the cost of her feed for the year.

DISCUSSION OF RESULTS

These records show a definite correlation between the feed cost and the annual butterfat production of a dairy cow. They show that this correlation exists in spite of a wide variation in the productive ability of the cows, the type of feed they are fed, the care and management they receive, the age of the cows and to a certain extent the market value of the feeds. These are true because of the great variation in the herds from which the data are collected and the six-year period over which the records extend.

The feed costs found in table 3 were obtained by use of the regression formula. These values are used because they represent the normal line of regression and vary but little from the actual feed cost in the various groups. This lack of any great deviation is shown in figure 3.

Data in table 3 show there is a smaller proportionate increase in feed cost when the production of a poor herd is doubled than when the production of a fair or good herd is doubled.

Use of the regression formula $x = 0.2453\ y + 36.68$ indicates that it costs an average of $36.68 a year for feed to maintain a dairy cow and additional feed worth $24.53 for each 100 pounds of butterfat she produces during the year. The probable explanation of this is that all cows are fed what hay and other roughage they will eat and are then fed grain in direct proportion to the amount of milk or butterfat they produce. This statement will also explain the smaller decrease in the feed cost of one

pound of fat, and the larger proportionate increase in annual feed cost when improvement is made in the better classes of dairy stock.

The very uniform and normal distribution of the data is indicated by the curves in figures 1 and 2 and by figure 3 which shows the close relation of the lines of regression and the distribution of the averages which determine these lines.

CONCLUSIONS

1. A positive correlation exists between the annual fat production and annual feed costs of dairy cows, under farm conditions
2. The feed cost of 1 pound of butterfat is lower when the annual production of a cow is greater.
3. This proportionate increase in feed cost is greater when the production of good cows is doubled, than when the production of poor cows is doubled.
4. These data indicate that cows require certain feed for maintenance, above which the feed cost for each hundred pounds of butterfat produced annually will be practically the same whether a cow is a light or heavy producer.
5. These data indicate that the high producing cow is more profitable because the cost of maintenance is spread over a larger quantity of product rather than because of a more effecient use of the feed she consumes.

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SOME OBSERVATIONS ON THE BASIC VISCOSITY OF ICE CREAM MIXES*

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In recent years the maximum viscosity of an ice cream mix has been termed apparent viscosity, and that viscosity which has been obtained by thorough agitation in mechanical devices, basic viscosity. Although this viscosity termed basic viscosity is the lowest viscosity attainable in a given ice cream mix under the conditions of agitation used, the question arises as to whether or not it is a true basic viscosity of the mix.

Some observations of fat clumps in thoroughly agitated ice cream mixes led to a further study of this problem. These studies tend to show that mechanical agitation, with air excluded, only partially breaks up the fat clumping in homogenized mixes and that these mixes have not attained their lowest possible viscosity for a given size of fat globule with this fat clumping present.

The clumping of fat globules in milk, cream and ice cream mixes has been shown by many investigators to increase the viscosity of these products. Likewise, two-stage homogenization of ice cream mixes has been shown to decrease fat clumping and reduce the viscosity of mixes.

Bateman and Sharp (1928) have published two important papers on the plasticity of milk and its products. They showed that milk, cream, and ice cream mixes were plastic and that even skimmilk was slightly plastic. They pointed out that since cream and ice cream mixes exhibit plasticity it is difficult to correlate single viscosity determinations with their other properties.

They showed that the repeated running of a sample of whole milk through the capillary tube reduced its viscosity and a microscopic study of this milk showed that this reduction in viscosity

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was related to the breaking up of the fat clusters. In a similar manner they found that the repeated running of skimmilk through the capillary tube did not reduce its viscosity.

Several noteworthy papers on basic viscosity as related to ice cream mixes have been published from the Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture. Leighton and Williams (1927) showed that two kinds of viscosity exist in an ice cream mix, namely, the apparent viscosity or that present after aging the mix without agitation and the viscosity which remains after the mix has been thoroughly agitated, which they termed basic viscosity. They agitated their mixes in a freezer with air excluded for a period of time which gave the maximum reduction in viscosity. For example, a mix containing 36.3 per cent total solids after one hour agitation reached a constant minimum viscosity which could not be reduced by continued agitation. In a later paper Leighton and Kurtz (1929) showed that these agitated mixes did not have plastic properties.

EXPERIMENTAL METHODS

Ice cream mixes for these experiments were standardized to contain 12 per cent fat, 10 per cent serum solids and 14 per cent sugar. They were pasteurized at 62.7° to 65.5°C. (145° to 150°F.) and homogenized at the pasteurization temperature and 2000 pounds pressure in a Manton-Gaulin homogenizer of 60 gallons per hour capacity. They were cooled over a surface tubular cooler to 4.4°C. (40°F.).

Twenty per cent serum solid mixtures were prepared with skimmilk and skimmilk powder, and processed the same as the ice cream mixes.

The viscosity determinations were made with the MacMichael viscometer using the disc bob. The viscosity was measured at a temperature of 2.0 ± 0.3°C. No endeavor was made to secure separate values for plasticity and viscosity.

A motor driven stirrer as described by Whitaker (1929) was used to thoroughly agitate the mixes. The mixes were agitated for twenty minutes with the stirrer, made of one-fourth inch wire

screening, revolving at 500 to 600 revolutions per minute. The apparatus provided for the exclusion of air during agitation.

The fat globules were measured at a magnification of approximately 2000 diameters using an ocular micrometer disc standardized with the microscope so adjusted that each of the smallest marks represented 0.625 micron. One-half cubic centimeter of the mix to be examined was diluted with 100 cc. of distilled water and mounted as a hanging drop preparation.

EXPERIMENTAL RESULTS

Two series of ice cream mixes containing butter, skimmilk, skimmilk powder, and sugar; and butter, skimmilk powder, water, and sugar which were prepared to study the effect of

TABLE 1

Viscosity in centipoises of mixes prepared with butter, skimmilk, skimmilk powder, sugar and gelatin homogenized at 2,000 pounds

MIX	APPARENT			AGITATED BASIC		
	0 hours aged	4 hours aged	20 hours aged	0 hours aged	4 hours aged	20 hours aged
1	3,000	3,930	5,360	182	174	249
2	2,020	4,860	5,830	158	163	242
3	227	1,270	1,880	108	120	137

aging on their properties have been included in these results. These mixes contained 0.56 per cent of a medium grade calf skin gelatin. In the first series viscosity determinations were made on the agitated and unagitated mixes. The results are given in table 1. As can be seen from table 1, the twenty minutes of agitation greatly reduced the viscosity of these very viscous mixes. The fat clumps, however, were only partially broken up by the agitation. This reduction in viscosity can be attributed to the breaking down of the gel structure and a partial splitting of the fat clumps.

In the next series of butter mixes a portion of the mix which had been homogenized at 2000 pounds pressure was run through the homogenizer four times at 200 pounds pressure to eliminate the

fat globule clumps as much as possible. This low pressure was chosen because it broke up fat clusters and it was assumed that such a low pressure would not alter other properties of the mix to any extent. There was, however, a slight difference in the size of individual fat globules in the 2000 and 200 pounds pressure mixes. Seven hundred individual globules in the 2000 pounds pressure mixes averaged 1.61 microns and 1000 individual globules in the 2000 and 200 pounds pressure mixes measured 1.40 microns. Viscosity determinations were made on these mixes as before.

TABLE 2

Viscosity in centipoises of mixes prepared with butter, skimmilk powder, sugar, water and gelatin

MIX	HOMOGENIZATION PRESSURE	APPARENT			AGITATED BASIC		
		0 hours aged	4 hours aged	20 hours aged	0 hours aged	4 hours aged	20 hours aged
1	2,000	1,270	5,830	7,500	139	129	125
	200*	129	1,010	1,050	68	98	120
2	2,000	640	1,760	3,680	96	87	113
	200*	106	880	1,310	65	86	118
3	2,000	221	1,830	2,800	60	105	99
	200*	77	680	960	51	70	74
4	2,000	1,520	3,600	4,870	103	133	179
	200*	55	560	930	45	57	83

* These mixes were homogenized at 2,000 pounds pressure and then four times at 200 pounds.

As is shown in table 2, this treatment greatly reduced the viscosity of these mixes in comparison with the portions of the mixes homogenized at 2000 pounds pressure. Also the viscosity of these mixes when agitated was less than the agitated mixes homogenized at 2000 pounds pressure. The presence of gelatin in these mixes introduced another factor which influenced their viscosity. An examination of these mixes under the microscope showed a direct relationship between the size of fat clusters and viscosity. Re-homogenization of the mix at 200 pounds pressure greatly reduced the original viscosity of the mix due to the split-

ting of fat clumps. The average size of fat clumps in mixes homogenized at 2000 pounds pressure was 7.55 by 4.11 microns and in the mixes re-homogenized at 200 pounds pressure 2.31 by 1.75 microns.

TABLE 3

Size of fat clumps in microns of mixes prepared with cream, skimmilk, skimmilk powder and sugar

MIX	HOMOGENIZATION PRESSURE	SIZE OF FAT GLOBULE CLUMPS	
		Apparent	Agitated basic
1	2,000	5.13 x 2.61	4.20 x 2.35
	200*	2.06 x 1.10	2.18 x 1.20
2	2,000	5.29 x 2.84	3.71 x 1.77
	200*	2.14 x 1.15	2.06 x 1.11
3	2,000	5.25 x 2.94	3.48 x 1.91
	200*	1.81 x 0.99	1.88 x 1.05

* These mixes were homogenized at 2,000 pounds pressure and then four times at 200 pounds.

TABLE 4

Viscosity in centipoises of mixes prepared with cream, skimmilk, skimmilk powder and sugar

MIX	HOMOGENIZATION PRESSURE	APPARENT		AGITATED BASIC	
		0 hours aged	20 hours aged	0 hours aged	20 hours aged
1	2,000	76.0	93.0	36.0	36.0
	200*	18.0	20.0	18.0	19.0
2	2,000		790.0	55.0	50.8
	200*		22.0		20.5
3	2,000	329.0	323.0	48.5	54.8
	200*	23.0	23.0	21.0	23.0

* These mixes were homogenized at 2,000 pounds pressure and then four times at 200 pounds pressure.

Another series of mixes standardized as before, were prepared without gelatin. Thirty per cent cream supplied the fat for these mixes in place of butter. These mixes were processed and viscosity determinations were made the same as in previous

experiments. One hundred fat clusters and individual globules found in the same fields were measured in each of these mixes before and after agitation. The results of these experiments are given in tables 3 and 4. The fat clumps of the mixes homogenized at 2000 pounds pressure were twice as large as the fat clumps found in these same mixes, after running them through the homogenizer four times at 200 pounds pressure. The viscosites of the former mixes were 4, 36 and 14 times the viscosities of the latter. Agitation reduced the viscosity of the mixes homogenized at 2000 pounds pressure to one-half, one-fifteenth and one-sixth of the original viscosity and cut the size of the clumps by approximately one miron from each dimension. Agitation of the mixes run through the homogenizer at 200 pounds pressure did not reduce their viscosity or alter the size of the fat clusters. The mixes homogenized at 2000 pounds pressure had a much smaller number of individual fat globules in proportion to clumps than when they were homogenized at 200 pounds pressure. The average size of 2000 individual globules was 1.47 microns. The agitated 2000 pounds pressure mixes were twice as viscous and contained fat clumps nearly twice as large as the agitated 200 pounds pressure mixes.

In a previous paper by the author (1928) measurements were made of a large number of fat globule clumps in ice cream mixes before and after freezing. The average size of clumps in mixes before freezing was 5.89 by 3.51 microns and after freezing 4.31 by 3.03. These mixes were homogenized with the single-stage valve. These results show the ineffectiveness of agitation in destroying fat globule clumps formed in homogenized mixes. It was also shown that agitation during freezing reduced the size of fat globule clumps to a greater extent in some mixes than in others.

The effect of agitation on mixtures with little or no clumping should give further information on the relation of clumps to changes in viscosity of agitated mixtures. Two different lots of milk containing 5.5 per cent fat were pasteurized at 65.5°C. (150°F.) and homogenized at 2000 pounds pressure at the pasteurization temperature. The clumps of fat globules in this

milk averaged 2.5 by 1.31 microns before agitation and after agitation 2.31 by 1.25 microns. The average viscosity of the two lots of milk at 0, 4, and 24 hour aging periods is as follows: 6.5, 6.25 and 6.00 centipoises. The viscosity of the agitated milk for the same periods is as follows: 6.66, 6.62 and 6.37 centipoises. There was practically no difference in the size of the clumps in the milk or the viscosity of the milk in either case.

An ice cream mix containing 10 per cent fat and 11 per cent serum solids was prepared as before except that it was homogenized with the two-stage valve at pressures of 3000 pounds on

TABLE 5

Viscosity in centipoises of 20 per cent serum solid mixes prepared with skimmilk and skimmilk powder

MIX	TREATMENT	APPARENT			AGITATED BASIC		
		0 hours aged	4 hours aged	20 hours aged	0 hours aged	4 hours aged	20 hours aged
1	Unhomogenized						
	Homogenized	10.9	11.9	14.0	10.9	12.5	14.0
2	Unhomogenized	11.0	13.0	15.0	10.0	12.8	14.9
	Homogenized	10.5	14.0	15.5	11.9	11.9	14.9
3	Unhomogenized	14.0	14.0	16.0	16.5	15.5	16.0
	Homogenized	11.0	14.0	14.5	13.3	14.8	16.0
4	Unhomogenized	12.0	13.0	14.5		16.2	15.0
	Homogenized	11.5	10.5	12.0	12.5	12.2	12.2

the first stage and 2200 on the second stage. Fourteen per cent sugar was added after homogenization. Fat globule clumping was almost eliminated but there were a few very small clumps present. The viscosity of this mix before agitation was 18.4 centipoises and 18.0 after agitation.

In another series of experiments skimmilk was standardized with skimmilk powder to contain 20 per cent serum solids without milk fat. These mixtures were pasteurized at 65.5°C. (150°F.) and half of each mix was homogenized at 2000 pounds pressure. Viscosity determinations were made on unhomog-

enized and homogenized samples both with and without agitation. The results are given in table 5. In some cases due to the agitating device not working properly foam occurred on some of the agitated 20 per cent skimmilk and skimmilk powder mixtures. These agitated samples increased slightly in viscosity likely due to the incorporation of a small amount of air. In two cases the agitated sample was slightly less viscous than the unagitated sample. In all other cases the agitated samples were practically the same or higher in viscosity than the unagitated samples. The serum solids mixes without fat could not be decreased in viscosity with agitation.

SUMMARY

These results tend to show that a particular method of mechanical agitation of ice cream mixes reduces their viscosity by a partial splitting of fat clumps, to a constant low viscosity, attainable under the conditions of agitation, but the size of clumps and the viscosity can be reduced still further by more vigorous mechanical treatment as when the mix is subsequently run through the homogenizer at low pressure.

The presence of clumps in ice cream mixes which have been agitated for basic viscosity determinations is a point which has apparently not been given serious consideration. Different conditions of agitation may vary in their effectiveness in splitting clumps and introduce a variable factor in the magnitude of the basic viscosity secured.

Basic viscosity, as the term is now used for ice cream mixes, applies to a value secured under specified conditions and it is not a correct minimum value from the viewpoint of the lowest viscosity that might be present without fat clusters.

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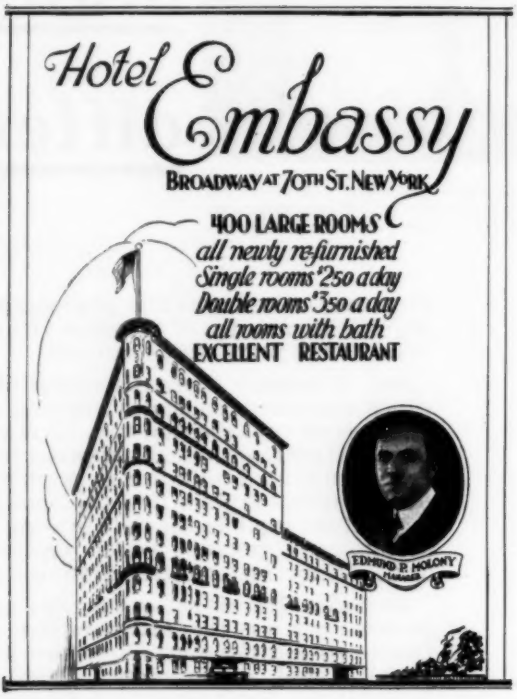

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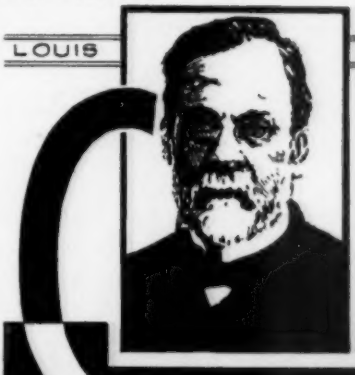

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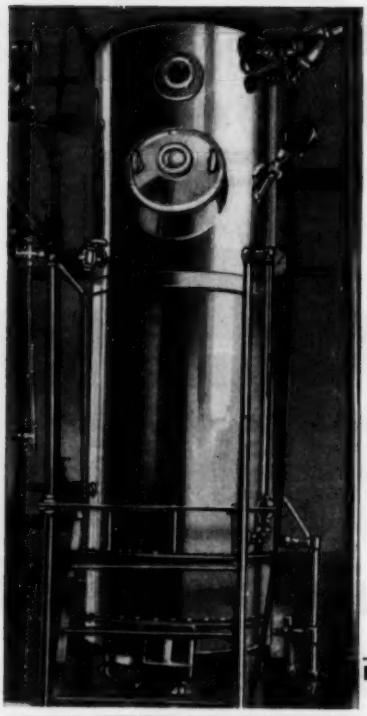

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